Dit proefschrift met stellingen van
ANNE WILLEM DE JONG
landbouwkundig ingenieur, geboren te
Semarang (Indonesië) op 26 november 1941,
is goedgekeurd door de promotor
Prof.Dr. J. Bruinsma, hoogleraar in de
plantenfysiologie aan de Landbouwhogeschool.

De Rector Magnificus van de Landbouwhogeschool,

Wageningen, 4 februari 1974 H.A. Leniger

PISTIL DEVELOPMENT IN CLEOME FLOWERS

A.W. de Jong

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD
VAN DOCTER IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF.DR.IR. H.A. LENIGER,
HOOGLERAAR IN DE TECHNOLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP
WOENSDAG 20 MAART 1974 DES NAMIDDAGS OM VIER UUR
IN DE AULA VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

STELLINGEN

I

Het optreden van afwisselend tweeslachtige en mannelijke bloemen aan de bloeiwijze van andromonoecische Cleome-soorten kan worden toegeschreven aan een tekort van cytokininen in de zich ontwikkelende bloemknoppen.

dit proefschrift, IV

II

Het is onjuist te beweren dat de stamperbevorderende werking van ethyleen een direkt effekt is.

Rudich, J., A.H. Halevy en N. Kedar: Plant Physiol. 50, 585-590, 1972; dit proefschrift, IV

III

De aborterende werking van gibberellinen op de stamper en op de zaadknop is van gelijke aard.

dit proefschrift, IV

IV

Voor de interpretatie van in vivo verkregen resultaten over de regulering van de bloemontwikkeling is de in vitro techniek onmisbaar.

V

Om de landbouw te concentreren op enkele gigantische gebieden is niet te realiseren.

Wassink, E.C.: Neth. J. agric. Sci. 18, 49-61, 1970

VI

Bij de huidige stand van mechanisatie van de landbouw is het toepassen van cytoplasmatische mannelijke steriliteit en fertiliteitsherstellende genen bij het produceren van hybride maïszaad een verouderde procedure.

VII

Aan tolerantie-veredeling wordt te weinig aandacht geschonken.

VIII

Hoewel vruchtdunningsmiddelen in de tuinbouw op grote schaal worden toegepast, is het werkingsmechanisme ervan nog niet bekend.

IX

De bakkwaliteit van tarwe wordt te eenzijdig beoordeeld op grond van het uiterlijk van de proefbroodjes.

INHOUD

KORTE SAMENVATTING

De bloemen van Cleome-planten ontwikkelen zich aan een andromonoecische tros. Dit wil zeggen dat er afwisselend tweeslachtige en mannelijke bloemen aan de bloeiwijze gevormd worden. Dit optreden van vrouwelijk geaborteerde bloemen is onderzocht door na te gaan wat de effekten zijn van minerale voeding en toediening van regulatoren en wat de invloed is van bladeren en vruchten.

Een hoog mineraal voedingsniveau begunstigt de stamperontwikkeling. De belangrijkste funktie van auxine hangt samen met de groei van de vruchten. Deze vruchten hebben een aanzienlijke "sink"-aktiviteit, waardoor voedings- en hormonale stoffen opgenomen kunnen worden. Gibberellinen aborteren de stampers en de zaadknoppen. Ethyleen stimuleert alleen maar de stamperontwikkeling in combinatie met gibberellinen. Deze gibberellinen kunnen worden verkregen uit andere plantendelen.

Om na te gaan wat de effekten zijn van voedings- en hormonale stoffen was het noodzakelijk bloemknoppen van Cleome iberidella op kunstmatige media te kweken. De kroonbladontwikkeling wordt bij afnemende concentratie van organische en anorganische voedingsstoffen altijd eerst geremd, terwijl de stamperontwikkeling nog optimaal blijft. Als dezelfde voedingsafhankelijkheid ook geldt voor Cleome spinosa in vivo is het onwaarschijnlijk dat de remming van de stamperontwikkeling toegeschreven kan worden aan voedingsgebrek. Bovendien blijkt dat cytokinine, i.c. zeatine en BA, noodzakelijk is om stampergroei te verkrijgen. Exogeen zeatine, toegediend aan intakte planten, onder voor de stampergroei ongunstige condities, herstelt de stamperontwikkeling.

De conclusie is dat het optreden van vrouwelijk geaborteerde bloemen het gevolg is van het tekort aan cytokininen in de bloemen boven de zich tegelijkertijd ontwikkelende vruchten, die een aanzienlijke concurrerende aktiviteit bezitten.

LIJST VAN GEBRUIKTE AFKORTINGEN EN PRODUKTNAMEN

ABA	abscisic acid
AMAB	allyl trimethylammonium bromide
BA	benzylaminopurine
CBBP	Phosphon-D, 2,4-dichlorobenzyl-tributyl-phosphonium chloride
Chlormequat	CCC, (2-chloroethyl)trimethylammonium chloride
Ethephon	(2-chloroethyl)phosphonic acid
GA	gibberellin
IAA	indoleacetic acid
2iP	isopentenylaminopurine,γ,γ-dimethylallyl-amonipurine
NAA	naphtaleneacetic acid
SADH	Alar, B-Nine, succinic acid-2,2-dimethyl-hydrazide

1. ALGEMENE INLEIDING

Het verschijnsel van de regulering van de ontwikkeling van de geslachtsorganen is één van de interessante aspecten van de bloemmorfogenese. De klassieke interpretatie van de bloem als een samengestelde as, waarbij de laterale leden in hun samenstelling vergelijkbaar zijn met een vegetatieve scheut, wordt algemeen toegeschreven aan Goethe (1831). De essentiële organen van de bloem zijn de meeldraden en de stampers, die direkt betrokken zijn bij het voortbestaan van de soort. De kelk- en kroonbladeren verrichten een secondaire functie.

Wanneer er zowel functionele meeldraden als vruchtbladen zijn, is de bloem tweeslachtig. Is daarentegen één van de geslachten afwezig of niet functioneel, dan is de bloem eenslachtig. Individuen van soorten kunnen óf bloemen voortbrengen van gelijk geslacht (monomorf), óf zij hebben bloemen van verschillend geslachtstype (polymorf). Volgens een recent overzicht van geslachtstypen bij Angiospermen (Heslop-Harrison, 1972) is Cleome andromonoecisch. Dit houdt in dat er tweeslachtige en mannelijke bloemen voorkomen op alle exemplaren van dezelfde soort.

Bij de bloemvorming zijn verschillende fasen te onderscheiden. Allereerst de overgang van een vegetatief in een generatief apicaal meristeem met de primordiën van de bloemaanleg. Daarna komen deze primordiën tot ontwikkeling, waarbij de differentiatie in functionele organen tot stand komt. De aanleg van de bloemorganen verloopt altijd van buiten naar binnen: kelk- en kroonbladeren, meeldraden en, vaak enige tijd later, de stamper. Heslop-Harrison (1963) heeft hiervoor een model ontwikkeld, waarbij een soort schakelsysteem achtereenvolgens de differentiatie van de bloemorganen reguleert. Deze differentiatie wordt geïnduceerd door specifieke genen of groepen van genen.

Onderdrukking of bevordering van geslachtsorganen kan optreden op elk niveau van de bloemvorming, zowel door milieu- als door andere faktoren, bv. door een wijziging van de hormonale balans in de buurt van de zich ontwikkelende primordiën.

Tegen deze achtergrond kan het hierna volgende onderzoek naar de stamperontwikkeling in Cleome-bloemen meer informatie verschaffen omtrent de hormonale regulatie van de sexualiteit in bloemen. We vinden immers mannelijke bloemen bij Cleome door stamperabortie. Door na te gaan welke faktor(en) deze stamperontwikkeling voortijdig doen afbreken, wordt een indruk verkregen van deze faktor(en).

2. OVERZICHT VAN REGULATOREN DIE BETROKKEN ZIJN BIJ DE GESLACHTS-EXPRESSIE VAN BLOEMEN

Napp-Zinn (1967) en Heslop-Harrison (1972) hebben een overzicht gegeven van de geslachtsbepaling bij planten.

Veel van het onderzoek naar de verandering van geslachtsexpressie is verricht met Cucurbitaceae. Een vervrouwelijkende werking van auxinen bij komkommer is het eerst waargenomen door Laibach & Kribben (1950a, b). Sindsdien is een zeer groot aantal publikaties verschenen met betrekking tot de vervrouwelijkende werking van auxinen in verscheidene plantensoorten: komkommer (Galun, 1959a, b), meloen (Brantley & Warren, 1960) en Cannabis sativa (Heslop-Harrison, 1956). Bij hop (Weston, 1960) en Mercurialis annua (Champault, 1969) treedt na auxinebehandeling vermannelijking op. Een hoog auxineniveau is geassocieerd met een vrouwelijke tendens bij komkommer (Galun et al., 1965; Rudich et al., 1972a) en maïs (Sladký, 1969).

Gibberellinen bevorderen vermannelijking in komkommerachtigen (Galun, 1959b; Peterson & Anhder, 1960; Wittwer & Bukovac, 1962; Pike & Peterson, 1969; Clark & Kenney, 1969; Krishnamoorthy, 1972) en andere soorten: Cannabis sativa (Herich, 1960; Köhler, 1964), Cosmos bipinnata (Rana & Jain, 1968) en gynoecische tomaat (Kubicki & Potaczek, 1972). Ook is wel vervrouwelijking door gibberellinen gevonden: Shifriss (1961) vond een toename van de vrouwelijke tendens bij Ricinus communis en Nelson & Rossman (1958) verkregen mannelijke steriliteit bij maïs.

De inductie van mannelijke bloemen aan vrouwelijke hennepplanten door gibberelline kan geheel of gedeeltelijk worden tegengegaan door abscisinezuur (ABA) (Mohan Ram & Jaiswal, 1972). De stimulering van vervrouwelijking door ABA is ook gevonden bij komkommerachtigen

(Abdel-Gawad & Ketellapper, 1969; Rudich et al., 1972a). Deze laatste auteurs vonden tevens een toename van het endogene ABA-niveau na Ethephonbehandeling (Ethephon stimuleerde de vervrouwelijking, zie hierna). Een hoog endogeen gibberellineniveau hangt samen met vermannelijking (Sladký, 1969, maïs; Nozzolillo, 1972, Fagopyrum esculentum; Atsmon et al., 1968, Hayashi et al., 1971, Hemphill et al., 1972, komkommer). Meer bewijs is hiervoor verkregen door het onderzoek van Chromińsky & Kopcewicz (1972), die aantoonden dat met Ethephon behandelde pompoenplanten eerst dan een verhoging van het endogene gibberellinegehalte te zien gaven, nadat de planten mannelijke bloemen begonnen te vormen. Ook de resultaten van Mitchell & Wittwer (1962) en Mishra & Pradhan (1970) ondersteunden het belang van het endogene gibberellineniveau bij de geslachtsbepaling van komkommer. Zij toonden aan dat de groeivertrager, Chlormequat en een modificatie ervan, AMAB, de vervrouwelijking bevorderden. Halevy & Rudich (1967) vonden eveneens een vervrouwelijking in meloen door middel van SADH. Een duidelijke uitspraak over het effekt van groeivertragers is afkomstig van Rudich et al. (1972b), die aantoonden dat in met SADH behandelde planten het endogene gibberellineniveau is afgenomen. In tegenspraak hiermee zijn de resultaten van Robinson et al. (1969), die geen effekten vinden van ABA, CBBP en Chlormequat op de geslachtsbepaling van komkommer. Wel is van ABA bekend dat het in staat is de gibberellinewerking te remmen (Chrispeels & Varner, 1966).

Het onderzoek naar het auxine- en gibberellinegehalte geeft een aanwijzing dat deze twee hormonen betrokken zijn bij de endogene regulatie van de geslachtsbepaling.

Ethyleenbehandeling stimuleerde de vervrouwelijking (Iwahori et al., 1970; Byers et al., 1972). In plaats van ethyleen wordt meestal Ethephon toegepast, een ethyleen vrijmakende stof (Yang, 1969; Warner & Leopold, 1969). Rudich et al. (1972a) vonden een ethyleentoename na Ethephonbehandeling van komkommerplanten. Een vervrouwelijkend effekt na Ethephonbehandeling is waargenomen bij Cucurbitaceae (McMurray & Miller, 1968; Rudich et al., 1969; Iwahori et al., 1969; Augustine et al., 1973) en andere planten (Kender & Remaily, 1970, druif; Mohan Ram & Jaiswal, 1970, Cannabis sativa). Robinson et al. (1970) demonstreerden bij wilde soorten van Cucurbita dat Ethephon niet de bloeminitiatie beïnvloedde, maar de ontwikkeling na de bloemaanleg.

Dat endogeen ethyleen betrokken is bij de geslachtsbepaling, is aangetoond door Rudich et al. (1972a). De auteurs vonden dat uit vrouwelijke komkommerlijnen meer ethyleen vrij kwam dan uit eenhuizige lijnen. Ook vonden zij dat planten, gekweekt onder korte dag - hetgeen vervrouwelijkend werkt - meer ethyleen ontwikkelden dan planten gekweekt onder lange dag. Verdere verduidelijking is gegeven door Byers et al. (1972), die vonden dat onder gereduceerde druk, als gevolg waarvan het endogene ethyleenniveau afneemt, een tendens naar vermannelijking optrad. Deze tendens was tegen te gaan door toediening van ethyleen.

In diverse systemen zijn tegengestelde effekten waargenomen van ethyleen en gibberellinen (Scott & Leopold, 1967; Russo et al., 1968). Volgens Robinson et al. (1969, komkommer) en Splittstoesser (1970, Cucurbita moschata) kan de door Ethephon geïnduceerde vrouwelijke bloei gedeeltelijk worden opgeheven door gibberellinebehandelingen. Sommige auxine-effekten, ook met betrekking tot de geslachtsbepaling in komkommer (Shannon & De La Guardia, 1969), worden bewerkstelligd door een ethyleenontwikkeling (Burg & Burg, 1967; Maxie & Crane, 1968). Dit stelt de hypothese discutabel dat faktoren die de geslachtsbepaling beïnvloeden, op de endogene balans van auxinen en gibberellinen inwerken. Hierbij tendeert een hoog auxineniveau naar vervrouwelijking, een hoog gibberellineniveau naar vermannelijking. De endogene auxineconcentratie kan de endogene ethyleenconcentratie reguleren. Het in vitro onderzoek van Galun et al. (1963) met bloemknoppen van komkommer bewijst echter hoe belangrijk de auxine/gibberelline balans is in de buurt van de zich ontwikkelende primordiën. Zij vonden dat een kleine hoeveelheid IAA (0.1 mg/l) reeds een vervrouwelijkend effekt heeft op mannelijke knoppen. Uit de literatuur (bv. Burg & Burg, 1967) is niet gebleken dat deze hoeveelheden auxine ethyleenvorming stimuleerde. Een bezwaar van het onderzoek van Galun et al. (1963) is, dat zij kinetine aan het medium hebben toegevoegd om verbleking van de kroonbladen tegen te gaan. Dit kinetine, eventueel bijgestaan door de lage IAAconcentratie, kan de vervrouwelijking hebben veroorzaakt.

Over de effekten van regulatoren als auxinen, gibberellinen, en ethyleen op de geslachtsbepaling is veel informatie beschikbaar. Dit is veel minder het geval met cytokininen. Tot nu toe is door cytokininebehandeling vervrouwelijking gevonden bij: Luffa acatangula (Bose & Nitsch, 1970), mannelijke bloemen van Vitis (Moore, 1970; Hashizume & Iizuka, 1971; Negi & Olmo, 1972), Bryophyllum crenatum (Catarino, 1964), Mercurialis soorten (Durand, 1967) en Melandrium dioicum (Nienhuis-Van Albada & Nigtevecht, 1968).

Toediening van groei-regulerende stoffen heeft niet uitsluitend een direkt effekt op de geslachtsbepaling van de bloemknop maar veeleer op de endogene hormoonbalans in de plant. Behandeling met groeistoffen verstoort de regulatie van vele processen in de plant. Een waargenomen effekt kan dus niet zonder meer als een direkt effekt worden verklaard. Om het inzicht in de regulering van de geslachtsbepaling te verdiepen, is het nodig om naast het onderzoek aan bloemknoppen in situ, ook de effekten van groeiregulatoren op bloemknoppen in vitro, als mede andere faktoren, te bestuderen. De eerste publikaties hierover zijn van Galun et al. (1962, 1963) en van Tepfer et al. (1962, 1963). Galun et al. (1963) toonden aan dat de door IAA geïnduceerde vervrouwelijking van potentiëel mannelijke bloemknoppen van komkommer door GA_3 kan worden opgeheven. Geïsoleerde tweeslachtige of vrouwelijke knoppen werden nauwelijks beïnvloed door IAA en GA_3. De knoppen kwamen tot het stadium dat het geslacht duidelijk kon worden waargenomen, maar volledige uitgroei van de knoppen tot bloem bleek niet mogelijk. Ook Tepfer et al. (1963) verkregen geen bloei met geïsoleerde knoppen van Aquilegia. Op een medium verrijkt met IAA, GA_3 en kinetine werden alleen de vruchtbladen - zij het zonder zaadknoppen - even groot als in vivo tijdens de bloei. De meeldraadgroei was slecht, alhoewel helmdraden en helmhokjes konden worden onderscheiden. Porath & Galun (1967) vonden pollenvorming in Cucumis melo bloemknoppen, maar de knoppen bereikten zelden het stadium waarin zaadknoppen konden worden waargenomen. Hetzelfde resultaat vermeldde Rodriques Pereira (1968) met Cucurbita pepo. Blake (1969) toonde aan dat zonder gibberelline vruchtbeginsel- en zaadknopontwikkeling werden geremd.

Mohan Ram & Wadhi (1968) verkregen geen volledige ontwikkeling van Kalanchoe pinnata bloemknoppen.

Verschillende faktoren zoals, licht, zuurgraad, minerale voeding, suikers, regulatoren en grootte en leeftijd van de explantaten, zijn van invloed op de orgaanontwikkeling in vitro (Nitsch & Nitsch, 1967a, b). Toch wordt veel onderzoek gedaan met bekende media zonder dat de minerale samenstelling hiervan wordt onderzocht. Zo ontbreekt bv. bij de media van Galun et al. (1963), Tepfer et al. (1963) en Mohan Ram & Wadhi (1968) gereduceerde stikstof.

De conclusie van dit literatuuroverzicht is dat er bezwaren zijn aan de interpretatie van effekten op de geslachtsexpressie van planten ten gevolge van behandeling met regulatoren. Deze behandeling houdt een verstoring van de endogene hormoonbalans in en heeft tevens gevolgen voor de regulatie van andere processen in de plant. Om direkte effekten op de uitgroei van bv. de stamper te onderscheiden van indirekte invloeden kan de in vitro kweek van bloemknoppen uitkomst bieden. Daarbij moet vooral ook aandacht besteed worden aan de samenstelling van het medium, welke namelijk voor elke plantensoort verschillend kan zijn.

3. PROBLEEMSTELLING

Soorten van het genus Cleome vertonen afwisselend zones met vruchten en zones zonder vruchten. In 1923 vermeldde Stout dat de onderbroken vruchtproduktie bij Cleome spinosa het gevolg is van onderdrukking van de stamperontwikkeling. Murneek (1927) vond dat verwijderen van stampers of jonge vruchten de vrouwelijke abortie in bovenliggende bloemknoppen doet afnemen. Hij suggereerde dat de verandering in geslachtsexpressie het gevolg is van de metabolische aktiviteit van de zich ontwikkelende vruchten. De vraag is daarbij in hoeverre de stamperontwikkeling beperkt wordt, niet alleen door voedingsfaktoren, maar ook door hormonale invloeden.

Bloei is de eindfase van de eenjarige plant. De gehele plant is betrokken bij de ontwikkeling van de bloemen. Het bestuderen van faktoren met betrekking tot de ontwikkeling van de stamper in de bloem is dan ook zeer complex doordat alle andere delen van de plant hun invloed daarop kunnen doen gelden.

In het voorafgaande is reeds aangeduid welke bezwaren kleven aan het onderzoek naar de geslachtsbepaling bij intakte planten. Daarom hebben wij enerzijds onderzoek gedaan aan intakte Cleome spinosa planten, anderzijds bloemknoppen geïsoleerd van Cleome iberidella en deze steriel gekweekt op kunstmatige media van bekende samenstelling. Deze Cleome iberidella is uit een grote collectie Cleome soorten geselecteerd omdat zij een identieke bloei, maar kleinere bloemen heeft, die zich derhalve goed lenen voor cultuur in vitro.

LITERATUUR

Abdel-Gawad, H.A. en H.J. Ketellapper: Regulation of growth, flowering and senescence of squash plants. II. Effects of 2-chloroethylphosphonic acid (Ethrel) and abscisic acid. Plant Physiol. 44, Suppl., 15, 1969.

Atsmon, D., A. Lang en E.N. Light: Contents and recovery of gibberellins in monoecious and gynoecious cucumber plants. Plant Physiol. 43, 806-810, 1968.

Augustine, J.J., L.R. Baker en H.M. Sell: Female flower induction on androecious cucumber, Cucumis sativus L. J. Amer. Soc. Hort. Sci. 98, 197-199, 1973.

Blake, J.: The effect of environmental and nutritional factors on the development of flower apices cultured in vitro. J. Exp. Bot. 20, 113-123, 1969.

Bose, T.K. en J.P. Nitsch: Chemical alteration of sex-expression in Luffa acutangula. Physiol. Plant. 23, 1206-1211, 1970.

Brantley, B.B. en G.W. Warren: Sex expression and growth in muskmelon. Plant Physiol. 35, 741-745, 1960.

Burg, S.P. en E.A. Burg: Auxin stimulated ethylene formation: Its relationship to auxin inhibited growth, root geotropism and other plant processes. In F. Wightman and G. Setterfield (Ed.) Biochemistry and Physiology of Plant Growth Substances. Ottawa, 1275-1294, 1967.

Byers, R.E., L.R. Baker, H.M. Sell, R.C. Herner en D.R. Dilley: Ethylene: A natural regulator of sex expression of Cucumis melo L. Proc. Nat. Acad. Sci. 69, 717-720, 1972.

Catarino, F.M.: Some effects of kinetin on sex expression in Bryophyllum crenatum Bak. Port. Acta Biol. A8, 267-284, 1964.

Champault, A.: Masculinisation d'inflorescences femelles de Mercurialis annua L. (2n=16), par culture in vitro, de noeuds isolés en présence d'auxines. C.R. Acad. Sc. Paris 269, 1948-1950, 1969.

Chrispeels, M.J. en J.E. Varner: Inhibition of gibberellic acid induced formation of α-amylase by abscissin II. Nature 212, 1066-1067, 1966.

Chromińsky, A. en J. Kopcewicz: Auxins and gibberellins in 2-chloroethylphosphonic acid-induced femaleness of Cucurbita pepo L. Z. Pflanzenphysiol. 68, 184-189, 1972.

Clark, R.K. en D.S. Kenney: Comparison of staminate flower production on gynoecious strains of cucumbers, Cucumis sativus L., by pure gibberellins (A_3, A_4, A_7, A_{13}) and mixtures. J. Amer. Soc. Hort. Sci. 94, 131-132, 1969.

Durand, B.: L'Expression de sexe chez Mercuriales annuelles. Bull. Soc. Franç. Physiol. Végét. 13, 195-202, 1967.

Galun, E.: The role of auxins in the sex expression in the cucumber. Physiol. Plant. 12, 48-61, 1959a.

- : Effects of gibberellic acid and naphtaleneacetic acid on sex expression and some morphological characters in the cucumber plant. Phyton 13, 1-8, 1959.
- S. Izhar en D. Atsmon: Determination of relative auxin content in hermaphrodite and andromonoecious Cucumus sativus L. Plant Physiol. 40, 321-326, 1965.
- Y. Jung en A. Lang: Culture and sex modification of male cucumber buds in vitro. Nature 194, 596-598, 1962.
- - - : Morphogenesis of floral buds of cucumber cultured in vitro. Develop. Biol. 6, 370-387, 1963.

Goethe, J.W. Von: Versuch über die Metamorphose der Pflanzen. Stuttgart, 1831.

Halevy, A.H. en Y. Rudich: Modification of sex expression in muskmelon by treatment with the growth retardant B-995. Physiol. Plant. 20, 1052-1058, 1967.

Hashizume, T. en M. Iizuka: Induction of female organs in male flowers of Vitis species by zeatin and dihydro zeatin. Phytochem. 10, 2653-2655, 1971.

Hayashi, F., D.R. Boerner, C.E. Peterson en H.M. Sell: The relative content of gibberellins in seedlings of gynoecious and monoecious cucumber (Cucumis sativus). Phytochem. 10, 57-62, 1971.

Herich, R.: Gibberellins and sex differentiation of flowering plants. Nature 188, 599-600, 1960.

Heslop-Harrison, J.: Auxin and sexuality in Cannabis sativa. Physiol. Plant. 9, 588-597, 1956.

- : Sex expression in flowering plants. Brookhaven Symp. Biol. 16, 109-122, 1963.
- : Sexuality of Angiosperms. In F.C. Steward (Ed.) Plant Physiology. A treatise VIC, 133-289, 1972.

Iwahori, S., J.M. Lyons en W.L. Sims: Induced femaleness in cucumber by 2-chloroethanephosphonic acid. Nature 222, 271-272, 1969.

- - en O.E. Smith: Sex expression in cucumber plants as affected by 2-chloroethylphosphonic acid, ethylene and growth retardants. Plant Physiol. 46, 412-415, 1970.

Kender, W.J. en G. Remaily: Regulation of sex expression and seed development with 2-chloroethylphosphonic acid. HortScience 5, 491-492, 1970.

Köhler, D.: Veränderung des Geschlechts von Cannabis sativa durch Gibberellinsäure. Ber. Deutsch. Bot. Ges. 77, 275-278, 1964.

Krishnamoorthy, H.N.: Effect of GA_3, GA_{4+7}, GA_5 and GA_9 on the sex expression of Luffa acatangula var. H-2. Plant and Cell Physiol. 13, 381-383, 1972.

Kubicki, B. en H. Potaczek: Gibberellin-induced perfect flowers in a tomato female form and the possibility of obtaining gynoecious lines for hybrid seed production. Genet. Pol. 13, 67-74, 1972.

Laibach, F. en F.J. Kribben: Der Einfluss von Wuchsstoff auf die Bildung männlicher und weiblicher Blüten bei einer monözischen Pflanze (Cucumis sativa L.). Ber. Deut. Bot. Ges. 62, 53-58, 1950a.

- - : Der Einfluss von Wuchsstoff auf das Geschlecht der Blüten bei einer monözischen Pflanze. Beitr. Biol. Pflanz. 28, 64-67, 1950b.

Maxie, E.C. en J.C. Crane: Effect of ethylene on growth and maturation of the fig, Ficus carica L., fruit.
Proc. Amer. Soc. Hort. Sci. 92, 255-267, 1968.

McMurray, A.L. en C.H. Miller: Cucumber sex expression modified by 2-chloroethanephosphonic acid. Science 162, 1397-1398, 1968.

Mishra, R.S. en B. Pradhan: The effect of (2-chloroethyl)trimethylammoniumchloride on sex expression in cucumber. J. hort. Sci. 45, 29-31, 1970.

Mitchell, W.D. en S.H. Wittwer: Chemical regulation of flower sex expression in Cucumis sativus L. Science 136, 880-881, 1962.

Mohan Ram, H.Y. en V.S. Jaiswal: Induction of female flowers on male plants of Cannabis sativa by Cepha. Experientia 26, 214-216, 1970.

- - : Induction of male flowers on female plants of Cannabis sativa by gibberellins and its inhibition by abscisic acid. Planta 105, 263-266, 1972.

- en M. Wadhi: Morphogenic potentialities of flower buds of Kalanchoe pinnata Pers. grown in vitro. Ann. Bot. 32, 825-832, 1968.

Moore, J.N.: Cytokinin-induced sex conversion in male clones of Vitis species. J. Amer. Soc. Hort. Sci. 95, 387-393, 1970.

Murneek, A.E.: Physiology of reproduction of horticultural plants. II. The physiological basis of intermittent sterility with special reference to the spider flower. Univ. Missoury Res. Bull. 106, 1972.

Napp-Zinn, K.: Modifikative Geschlechtsbestimmung bei Spermatophyten. In W. Ruhland (Ed.) Handbuch der Pflanzenphysiologie 18. Berlin-Heidelberg-New York, 153-213, 1967.

Negi, S.S. en H.P. Olmo: Certain embryological and biochemical aspects of cytokinin SD 8339 on converting sex of a male Vitis vinifera (sylvestris). Amer. J. Bot. 59, 851-857, 1972.

Nelson, P.M. en E.C. Rossman: Chemical induction of male sterility in inbred maize by use of gibberellins. Science 127, 1500-1501, 1958.

Nienhuis-van Albada, J.A. en G. van Nigtevecht: Geslachtsbeïnvloeding door middel van een synthetische cytokinine bij Melandrium dioicum. Genen en Phaenen 12, 6-10, 1968.

Nitsch, C. en J.P. Nitsch: The induction of flowering in vitro in stem segments of Plumbago indica L. I. The production of vegetative buds. Planta 72, 355-370, 1967.

- - : The induction of flowering in vitro in stem segments of Plumbago indica L. II. The production of reproductive buds. Planta 72, 371-384, 1967.

Nozzolillo, C.: The effect of gibberellic acid on buckwheat flowers. Can. J. Bot. 50, 901-904, 1972.

Peterson, C.E. en L.D. Anhder: Induction of staminate flowers on gynoecious cucumbers with gibberellin A_3. Science 131, 1673-1674, 1960.

Pike, L.M. en C.E. Peterson: Gibberellin A_4/A_7, for induction of staminate flowers on the gynoecious cucumber. Euphytica 18, 106-109, 1969.

Porath, D. en E. Galun: In vitro culture of hermaphrodite floral buds of Cucumis melo L.: microsporogenesis and ovary formation. Ann. Bot. 31, 283-290, 1967.

Rana, R.S. en H.K. Jain: Gibberellin induced expression of male potential in a stamenless mutant of Cosmos. Naturwiss. 55, 301-302, 1968.

Robinson, R.W., S. Shannon en M.D. De La Guardia: Regulation of sex expression in cucumber. Bio Sci. 19, 141-142, 1969.

- , T.W. Whitaker en G.W. Bohn: Promotion of pistillate flowering in Cucurbita by 2-chloroethylphosphonic acid. Euphytica 19, 180-183, 1970.

Rodrigues Pereira, A.S.: Sex expression in floral buds of Acorn squash. Planta 80, 349-358, 1968.

Rudich, J., A.H. Halevy en N. Kedar: Increase in femaleness of three cucurbits by treatment with Ethrel, an ethylene releasing compound. Planta 86, 69-76, 1969.

- - - : Ethylene evolution from cucumber plants as related to sex expression. Plant Physiol. 49, 998-999, 1972.

- - - : The level of phytohormones in monoecious and gynoecious cucumbers as affected by photoperiod and Ethephon. Plant Physiol. 50, 585-590, 1972.

- - - : Interaction of gibberellin and SADH on growth and sex expression of muskmelon. J. Amer. Soc. Hort. Sci. 97, 369-372, 1972.

Russo, L., H.C. Dostal en A.C. Leopold: Chemical regulation of fruit ripening. Bio Science 18, 109, 1968.

Scott, P.C. en A.C. Leopold: Opposing effects of gibberellin and ethylene. Plant Physiol. 42, 1021-1022, 1967.

Shannon, S. en M.D. De La Guardia: Sex expression and the production of ethylene induced by auxin in the cucumber (Cucumis sativus L.). Nature 223, 186, 1969.

Shiffriss, O.: Gibberellin as sex regulator in Ricinus communis. Science 133, 2061-2062, 1961.

Sladký, Z.: Role of growth regulators in differentiation processes of maize (Zea Mays L.) organs. Biol. Plant. 11, 208-215, 1969.

Splittstoesser, W.E.: Effects of 2-chloroethylphosphonic acid and gibberellic acid on sex expression and growth of pumpkins. Physiol. Plant. 23, 762-768, 1970.

Stout, A.B.: Alternation of sexes and intermittent production of fruits in the spider flower (Cleome spinosa). Amer. J. Bot. 10, 57-66, 1923.

Tepfer, S.S., R.J. Greyson, W.R. Craig en J.L. Hindman: In vitro culture of floral buds of Aquilegia. Amer. J. Bot. 50, 1035-1045, 1963.

- - en J.L. Hindman: In vitro culture of floral buds of Aquilegia. Amer. J. Bot. 49, 657, 1962.

Warner, H.L. en A.C. Leopold: Ethylene evolution from (2-chloroethyl)-phosphonic acid. Plant Physiol. 42, 156-158, 1969.

Weston, E.W.: Changes in sex in the hop caused by plant growth substances. Nature 188, 81-82, 1960.

Wittwer, S.H. en M.J. Bukovac: Staminate flower formation on gynoecious cucumbers as influenced by the various gibberellins. Naturwiss. 13, 305-306, 1962.

Yang, S.F.: Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol. 44, 1203-1204, 1969.

PISTIL DEVELOPMENT IN *CLEOME* FLOWERS. I. EFFECTS OF NUTRIENTS AND OF THE PRESENCE OF LEAVES AND FRUITS ON FEMALE ABORTION IN *CLEOME SPINOSA* JACQ.

A.W. de Jong and J. Bruinsma

Department of Plant Physiology,
Agricultural University, Wageningen

Accepted for publication in
Zeitschrift für Pflanzenphysiologie

Department of Plant Physiology, Agricultural University, Wageningen

Pistil development in Cleome flowers. I. Effects of fertilization and of the presence of leaves and fruits on female abortion in Cleome spinosa Jacq.

A.W. de Jong and J. Bruinsma

With 3 figures

SUMMARY

Pistil development in flower buds of Cleome spinosa Jacq. is stimulated by fertilization and the presence of mature leaves. Young developing fruits at the inflorescence inhibit pistil development in the flower buds on top. The inhibition coincides with and is partially preceded by a sharp rise in the IAA content of the seeds, which probably affects pistil growth by inducing a competing growth of the fruits. Gibberellins, accumulating towards fruit maturation, play no appreciable role in the effect of fruits on pistil development.

INTRODUCTION

Several species of the genus Cleome show an alternation of fruited and sterile zones at the part of the inflorescence which is out of flowers. In 1923 Stout reported alternating suppression of pistil development in zones of flowers of Cleome spinosa as the cause of this intermittent fruit production. Murneek (1927) found that removal of young fruits resulted in a decrease of female abortion, the effect being even more pronounced when pistils were removed. He suggested this change in sex expression to be caused by increased metabolic activity of developing fruits. Joshi (1939) obtained similar results with the unisexual flowers of Musa paradisiaca: removal of female flowers or fruits allowed for the development of further female instead of male flowers. He concluded that sex expression depends on the available amount of nutrients and that by removal of fruits the nutritive level is increased. It

may be questioned, however, as to which extent pistil development is limited not only by nutritive factors, but also by hormonal substances, either produced or attracted by developing fruits.

In a first attempt to distinguish between the contribution of nutritive and hormonal factors to the pistil development in flower buds, the effects of fertilization and of the presence of leaves and fruits were analysed. The hormonal contents of fruits were also taken into account.

MATERIAL AND METHODS

Clones of cuttings of spider flower, Cleome spinosa Jacq.[1] cv. "Pink Queen", were grown in 4 l plastic pots in a greenhouse at a day length of at least 16 hrs. Part of them were transferred to the field, the rows were spaced 80 cm apart, the distance between plants within the rows being 30 cm. All lateral shoots were removed to obtain main axes only.

In the experiments on leaf effects, all leaves except the upper or lower six were cut away about two weeks before bloom. In the experiments on fruit effects, all fruits were removed every third day. The newly formed flowers were counted daily and their sex established.

The spectrofluorometric determination of indoleacetic acid (IAA) was carried out according to Knegt and Bruinsma (1973), gibberellin determinations were performed by amylase bioassay after van Loon and Bruinsma (in preparation).

RESULTS

Table 1 represents the effect of fertilization with extra N, P and K (12:10:18) on flower production and female abortion. The observations were continued until 3 weeks after the last treatment. Increasing the level of nutrition caused a decrease of female abortion, although the total number of flowers increased, together with the size of the inflorescence. The larger number of fruits produced and the increase in fruit weight at the highest fertilization level caused an increase of total fruit weight per plant with 75 per cent. Nevertheless, female abortion was clearly found to be

[1] Breteler 7307, Herbarium Vadense (WAG) at Wageningen

Table 1: Effect of 2, 4 and 6 ng NPK (12:10:18), applied 3 times during 6 weeks, on pistil development of flowers at Cleome spinosa main inflorescences.

	g NPK per plant				
	0	2	4	6	LSD (5%)
% female abortion	31.5	22.1	23.5	20.3	6.5
total No. of flowers	221	225	231	253	16
inflorescence length (cm)	48	53	56	62	7

Table 2: Effect of removal of all developing fruits, their distal halves, or their seeds, 0, 10 or 21 days after pollination, on female abortion. Numbers followed by different indices differ significantly at P = 0.05.

removal ... days after pollination	per cent female abortion			
	removal of			
	whole fruits	half fruits	seeds	control
0	0.5 a	0.5 a	0.1 a	
10	6.8 b	13.4 c	14.6 c	19.7 de
21	19.4 de	16.3 cd	21.3 e	

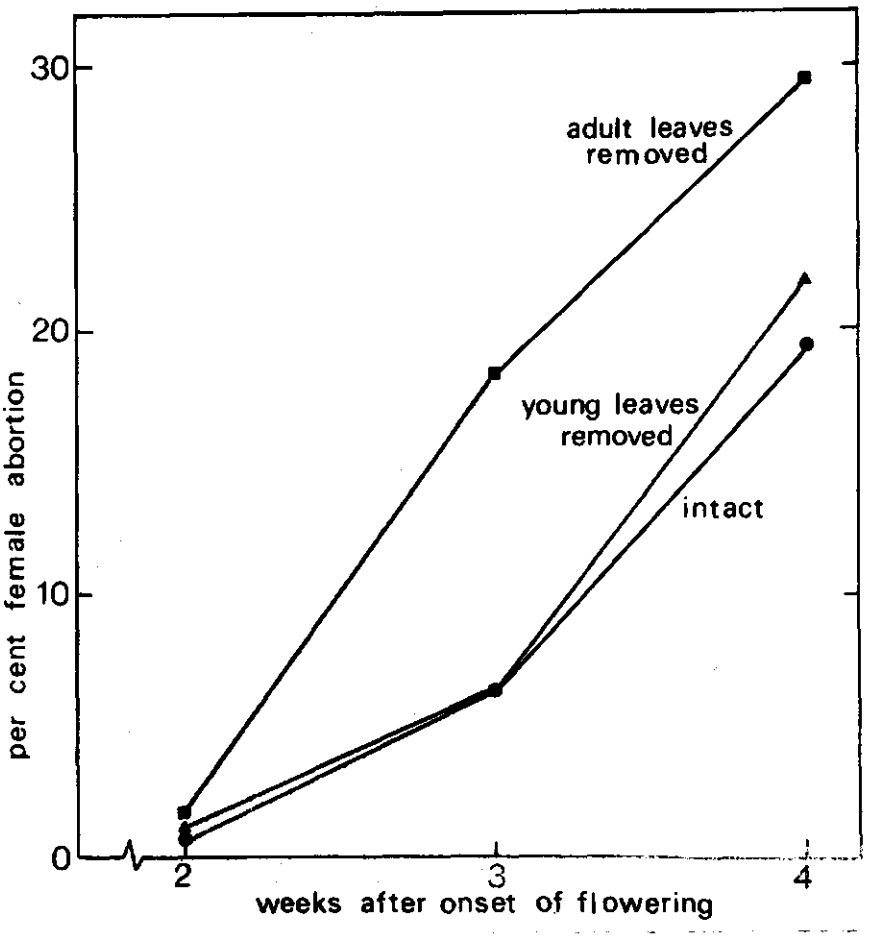

Fig. 1:
Effect of leaf removal on pistil development of Cleome spinosa flowers. All leaves, except the six most mature or juvenile ones, were removed 2 weeks before flowering. Observations continued 4 weeks following the onset of flowering. LSD at P = 0.05 is 8.2%.

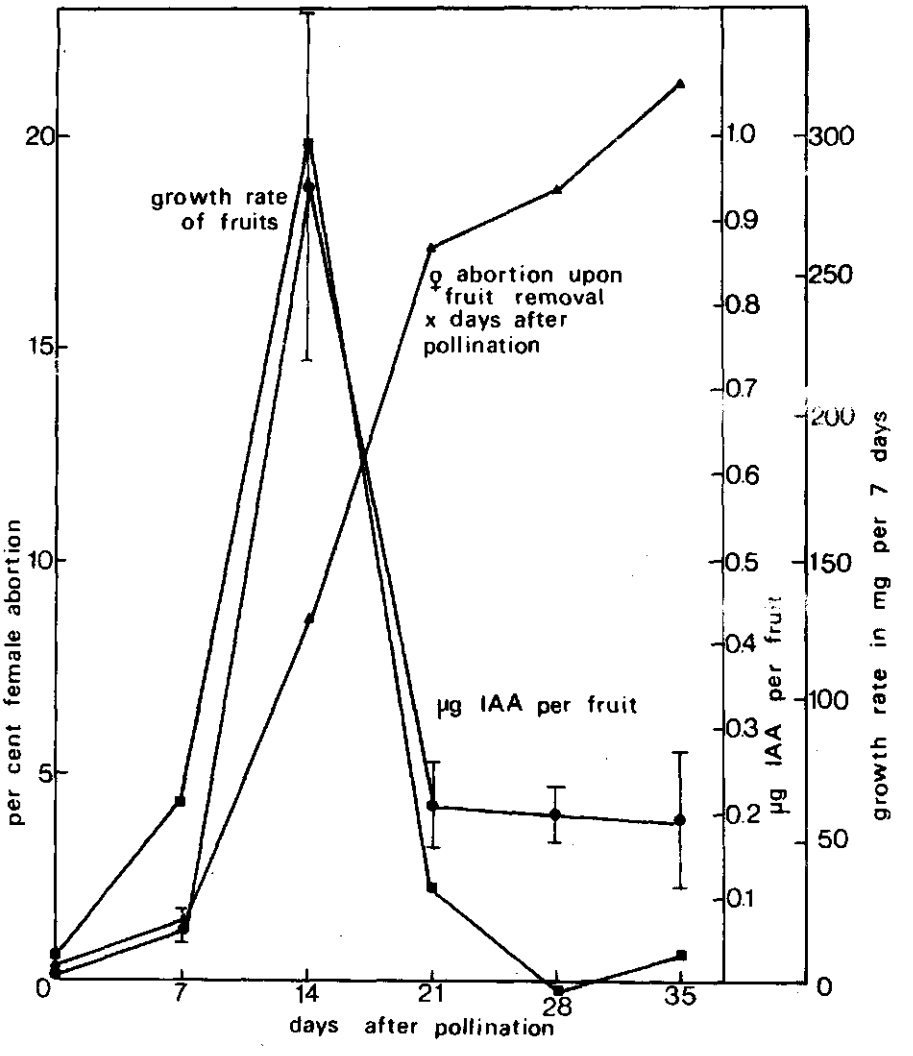

Fig. 2:
Growth rate and IAA-content per fruit and effect of fruit removal on female abortion.

diminished.

Removal of the young leaves two weeks before bloom, leaving the six most mature ones on the axis only, had no significant effect on pistil development (Fig. 1). On the contrary, removal of the full-grown leaves, leaving the six most juvenile only, considerably inhibited pistil development. After 4 weeks the difference between removal of juvenile and adult leaves diminished. Further experiments revealed that after this period only the effect of leaf removal remained, without a difference between removal of young or mature leaves, because all the leaves were full-grown after this interval. There was no significant effect of the treatments on the growth of the inflorescence (10 to 11 cm per week) or the number of flowers (4.6 per plant per day).

Following the early observations of Murneek (1927), the influence of growing fruits on pistil development in the superior flower buds was examined. Table 2 shows that removal of ovaries or ovules at anthesis prevented female abortion almost completely. Removal 10 days after pollination also reduced female abortion, the effect of seed removal being less pronounced than that of the removal of whole fruits. However, the difference between the effects of seed and fruit removal after 10 days was not always found to be significant, the major inhibition of pistil growth originating from the seeds rather than from the carpels. After 21 days, fruit removal no longer affected the growth of the pistils in the flower buds on top, the whole period of fruit development being about 30 days.

Fig. 2 presents the effect of the removal of fruits at different days after pollination upon female abortion, together with the growth rate and the contents of IAA of the fruits. It is clearly shown that fruit growth and IAA-content are closely correlated and that the effect on pistil development is largest at and shortly after the optimum fruit growth.

The growth rate of the seeds closely parallels that of the carpels (Fig. 3), the IAA in the fruits being nearly completely restricted to the seeds. Whereas the course of the auxin content follows an optimum curve parallel to the growth rate, the content of gibberellins, expressed as GA_3-equivalents, shows a steep increase only after attaining the maximum weight (Table 3).

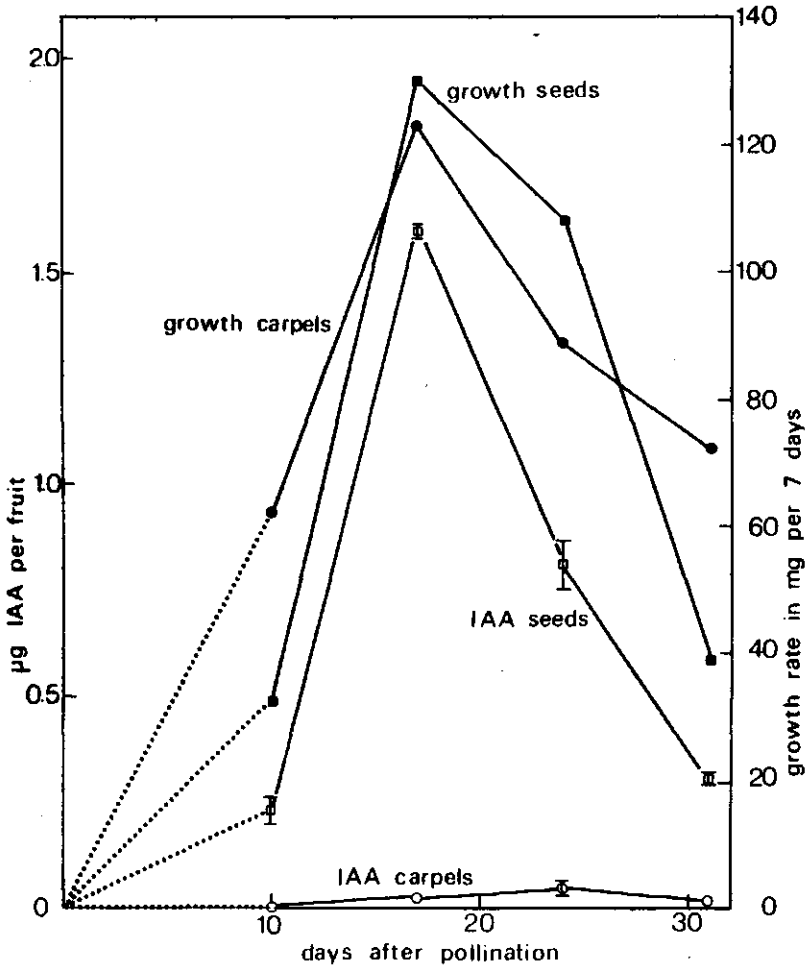

Fig. 3: Growth rate and IAA-content of seeds and carpels.

Table 3: Gibberellin contents of developing fruits.

days after pollination	0	7	14	21	28	35
fruit weight (mg)	8.8	75.5	373.6	406.5	404.1	414.2
ng GA_3-eq. per fruit						(ripe)
exp. 1	1.5	2.0	28.7	450	484	1022
exp. 2	1.3	10.0	31.2	147	328	2478

DISCUSSION

The pistil development in flower buds may depend on nutritive and hormonal factors, the latter possibly being partly stimulatory, partly inhibitory to pistil growth. The generally beneficial effect of nutrients on flower development (Napp-Zinn, 1967) is also demonstrated in the present paper. A high level of mineral fertilization promotes both the number of flowers produced and the development of pistils in these flowers (Table1). The presence of mature leaves, exporting photosynthetic assimilates, is also promotive of pistil growth, which is thus stimulated by both mineral and organic nutrients.

In our experiments, removal of young leaves had no effect on pistil growth. Galun (1959) found that removal of young cucumber leaves promoted the male tendency and removal of old leaves stimulated feminization; sex expression with these unisexual flowers is, however, largely determined by hormonal factors, and Galun explained his results by assuming auxin production of young leaves furthering the female tendency. This effect of young leaves was absent in our experiments.

The main factor influencing pistil growth is the development of young fruits at the inflorescence underneath the flower buds (Table 2). It is generally accepted that developing fruits act as a sink by the hormone production of their seeds (Crane, 1964; Seth and Wareing, 1967; Varga and Bruinsma, 1973). In Cleome spinosa fruits, auxin and gibberellin accumulate, the former mainly during the second week of development (Fig. 2), the latter gradually towards the end of fruit maturation (Table 3). By the time of this gibberellin accumulation, the effect of the presence of fruits or seeds on pistil development has passed (Table 2, Fig. 2), so that pistil abortion cannot be ascribed to the gibberellin production of the fruits. The rise in IAA-level of fruits, on the contrary, precedes and partly coincides with this effect. Therefore, pistil abortion can be ascribed to this auxin production, either by a direct inhibitory effect of IAA on pistil growth or by creating a sink activity for nutritive and, possibly, other hormonal factors as reflected by the concurrent fruit growth. The former possibility is less probable because auxins are generally promoting rather than inhibiting pistil

development and, moreover, IAA is unlikely to be translocated acropetally from the fruits to the superior flower buds. In addition, the benificial effects of mineral and organic nutrients on pistil development may well point to competition between developing fruits and flower buds on the inflorescence. Therefore, pistil abortion in flower buds of Cleome spinosa is tentatively ascribed to the sink activity of subtending developing fruits, due to the auxin production of their seeds.

ACKNOWLEDGEMENTS

We wish to thank Miss A.C. Burgers and Mrs. J.H. IJff for their technical assistance. This investigation was supported by the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

REFERENCES

Crane, J.C.: Growth substances in fruit setting and fruit development. Annu.Rev.Plant Physiol. 15, 303-326 (1964).

Galun, E.: The role of auxins in the sex expression of the cucumber. Physiol.Plant. 12, 48-61 (1959).

Joshi, A.C.: A note on the determination of sex of Plantain flowers. J.Genetics 38, 353-356 (1939).

Knegt, E. and J. Bruinsma: A rapid, sensitive and accurate determination of indolyl-3-acetic acid. Phytochem. 12, 753-756 (1973).

Murneek, A.E.: Physiology of reproduction in horticultural plants. II. The physiological basis of intermittent sterility with special reference to the Spider Flower. Univ. of Missouri, Res.Bull. 106, 37 pp. (1927).

Napp-Zinn, K.: Modifikative Geschlechtsbestimmung bei Spermatophyten. In W.Ruhland (Ed.) Handbuch der Pflanzenphysiologie 18. Berlin-Heidelberg-New York, 153-213 (1967).

Seth, A. and P.F. Wareing: Hormone-directed transport of metabolites and its possible role in plant senescence. J.Exp.Bot. 18, 65-77 (1967).

Stout, A.B.: Alternation of sexes and intermittent production of fruit in the Spider Flower (Cleome spinosa). Amer.J.Bot. 10, 57-66 (1923).

Varga, A. and J. Bruinsma: The growth and ripening of tomato fruits at different levels of endogenous cytokinins. J.Hort.Sci., in press (1973).

Ir. A.W. de Jong and Prof.Dr. J. Bruinsma, Department of Plant Physiology, Agricultural University, Arboretumlaan 4, Wageningen, The Netherlands.

PISTIL DEVELOPMENT IN CLEOME FLOWERS. II. EFFECTS OF NUTRIENTS ON FLOWER BUDS OF CLEOME IBERIDELLA WELW. EX OLIV. GROWN IN VITRO

A.W. de Jong, A.L. Smit and J. Bruinsma

Department of Plant Physiology,
Agricultural University, Wageningen

Accepted for publication in
Zeitschrift für Pflanzenphysiologie

Department of Plant Physiology, Agricultural University,
Wageningen

Pistil development in Cleome flowers. II. Effects of nutrients on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro.

A.W. de Jong, A.L. Smit and J. Bruinsma

With 8 figures

SUMMARY

Effects were studied of nutritive factors and their interaction with growth substances on bud and pistil development of Cleome iberidella Welw. ex Oliv. flower buds grown in vitro. Petal growth is more sensitive to carbohydrate and nitrogen deficiencies than pistil growth. Pistil development is favoured by unusually low pH-values. Data for an improved medium for floral organ culture are presented.

INTRODUCTION

Pistil development in Cleome spinosa flowers is affected by the presence of leaves and fruits, which produce and consume nutritive and hormonal substances (de Jong and Bruinsma, 1974a). In order to study the proper effects of these substances in flower morphogenesis, they have to be added in artificial media of known composition to isolated flower buds grown in vitro culture. Because of its large flowers, Cleome spinosa is not suitable for this purpose. From a large number of Cleome speciès, Cleome iberidella Welw. ex Oliv. was selected for its similar habit of pistil development with flowers of only about 13 mm length. Effects of nutritive factors on bud and pistil growth, and their interaction with some growth regulators, were studied.

MATERIAL AND METHODS

Clones of cuttings of Cleome iberidella Welw. ex Oliv.[1] were grown in a greenhouse at a daylength of at least 16 hrs. Flower buds were taken from young inflorescences only. After cutting, up to 100 flower

[1] Breteler 7305, Herbarium Vadense (WAG) at Wageningen

buds were sterilized in 200 ml 1.5% NaOCl and 0.005% Triton X-100 for 1 min. and rinsed with 700 ml sterilized water. Ten buds were inoculated on 25 ml medium in a 100 ml erlenmeyer, one treatment consisting of 4 replicates. The basal medium (BM) used is presented in Table 1; it was enriched with 10^{-6}M zeatin and sometimes with 10^{-7}M 1-naphtylacetic acid (NAA) (de Jong and Bruinsma, 1974b). Thermolabile compounds were added to the media after sterilization through a Millipore filter (pore size 0.45 µm).

The flower buds were grown during 14 days at 16 hrs at 22°C, illuminated by fluorescent and incandescent lamps (red light 0.072, blue light 0.085 Watt.m^{-2}.nm^{-1}), and 8 hrs at 18°C in the dark, at a relative air humidity of about 70%.

The lengths of the bud and of the gynophore plus pistil were measured to 0.1 mm using a dissecting microscope. From the measurements average values and the least significant difference (LSD) at P = 0.05 were computed.

RESULTS

By measuring a large variety of flower buds grown on the plants, ranging from 1 to 11 mm length, and plotting the bud length against the length of the gynophore + pistil, two regression lines were obtained: one for perfect and one for male flowers (Figs. 1 and 2). From these two lines the critical phase of pistil development turns out to be at about 2.3 mm bud length.

Effect of bud size. Table 2 shows that optimum pistil growth is obtained with flower buds of about 2.7 mm length, which have passed the critical phase already. Therefore, flower buds of about 2.5 mm length were used, sampled from young inflorescences only, because the first few flowers are always perfect.

Effect of acidity. Cultures in vitro of flowers or floral organs have generally been performed at a pH of 5.5 to 5.8 (e.g., Peterson, 1973). Fig. 3 shows, however, that pistil development, particularly, is favoured at lower pH-values. In further experiments a pH 5.0 was used for the media. During the 14 days of culture in vitro no appreciable shift in acidity occurred.

Effect of carbohydrates. Sucrose is a suitable sugar to enable growth of the flower bud (Fig. 4). The effect of increasing sugar

Table 1: Composition of the basal medium (BM), in mg/l, according to C. Nitsch, personal communication, 1971.

macro-elements:		FeEDTA:	
KNO_3	2000	5 ml/l of a solution containing	
NH_4NO_3	1500	Na_2EDTA	7450
$CaCl_2.2H_2O$	400	$FeSO_4.7H_2O$	5570
$MgSO_4.7H_2O$	400		
KH_2PO_4	200	amino acids and vitamins:	
		glycine	2
micro-elements:		myo-inositol	100
$MnSO_4.4H_2O$	25	nicotinic acid	5
H_3BO_3	10	pyridoxin HCl	0.5
$ZnSO_4.7H_2O$	10	thiamin HCl	0.5
$Na_2MoO_4.2H_2O$	0.25	biotin	0.05
$CuSO_4.5H_2O$	0.025	folic acid	0.5
		sucrose	30,000
		agar	8,000

Table 2: Effect of bud size on bud and pistil growth in vitro during 14 days om BM + 10^{-6}M zeatin.

bud length at cutting (mm)	1.4±0.1	1.8±0.1	2.3±0.1	2.7±0.1	3.4±0.3	LSD (5%)
bud length (mm)	5.90	6.39	7.50	7.86	9.13	0.67
length of gynophore + pistil (mm)	2.23	2.99	4.04	5.80	3.20	0.82
% perfect flowers	36	52	70	83	36	--

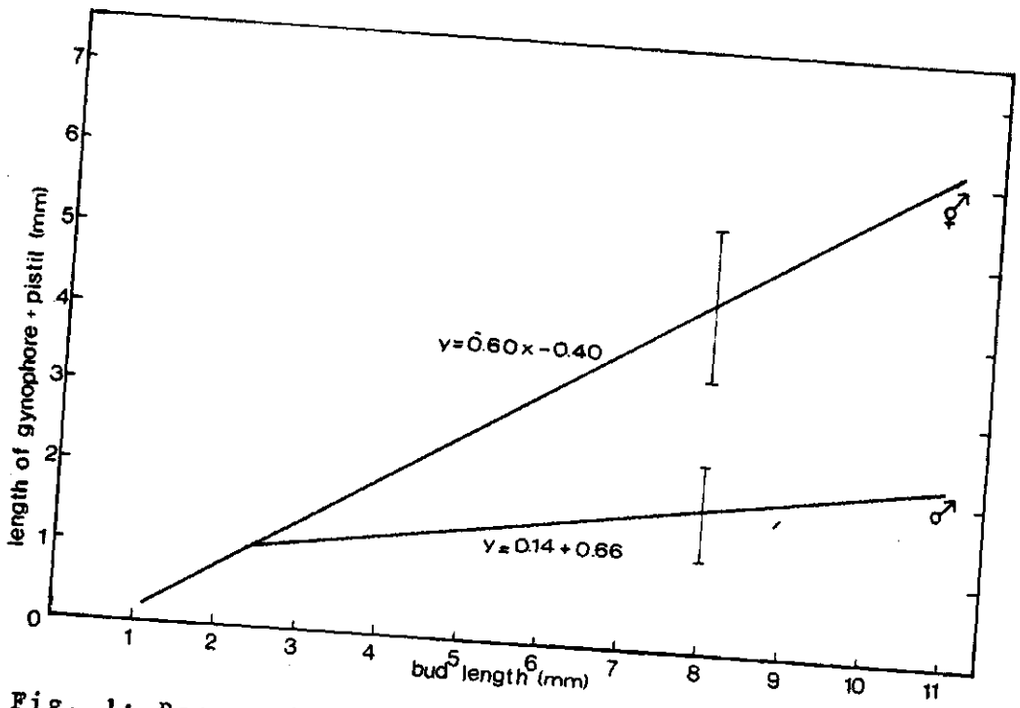

Fig. 1: Regression lines for perfect and male flower buds, grown in vivo. The twofold standard error of correlation coefficient is indicated.

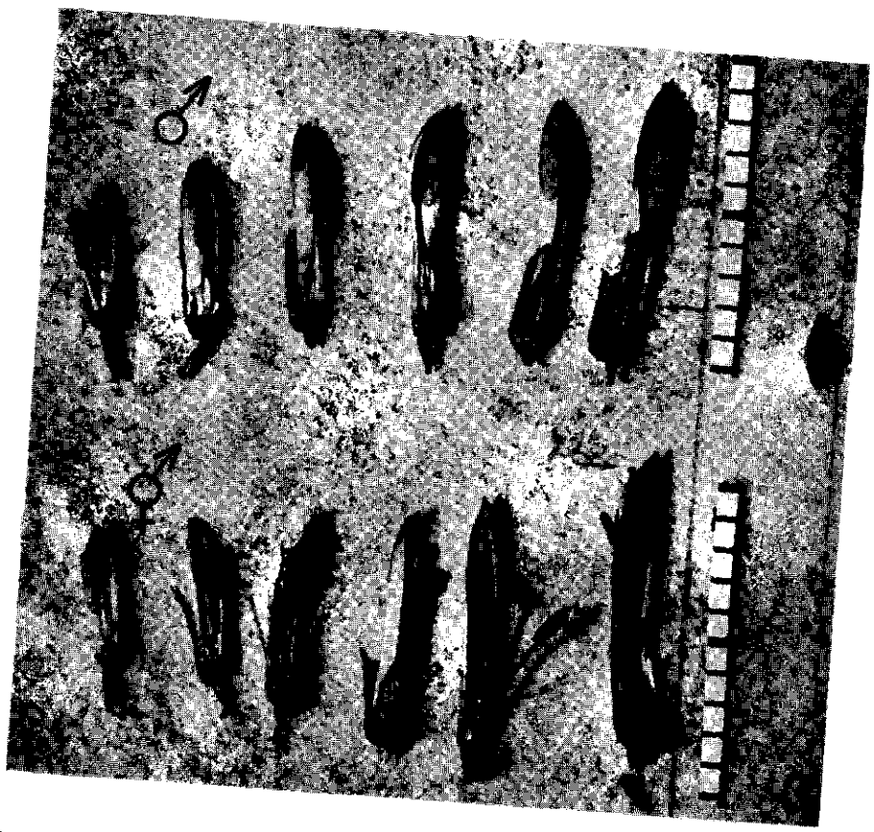

Fig. 2: Different stages of perfect and male flower buds grown in vivo. Arrows indicate the stigma. Scale in mm. At right a flower bud as used in the culture in vitro.

Table 3: Effect of organic nitrogen compounds on the development of flower buds grown on BM + 10^{-6}M zeatin + 10^{-7}M NAA during 14 days.

		length of bud and gynophore + pistil (mm)				
concentration (mg/l)		0	10	50	250	LSD (5%)
casein hydrolysate	bud	6.62	7.03	7.17	7.35	0.65
	pistil	3.01	2.45	2.44	2.83	n.s.[x]
yeast extract	bud	7.41	7.16	7.02	6.42	0.52
	pistil	2.63	2.16	2.19	1.75	0.33
glutamine	bud	8.29	8.22	8.54	8.17	n.s.
	pistil	4.56	3.91	3.87	4.25	n.s.
urea	bud	8.08	7.57	7.33	6.76	0.74
	pistil	4.92	4.54	3.42	2.44	1.08

[x] n.s. = not significant

Table 4: Effect of nitrogen compounds, added in equimolar amounts to nitrogen-free BM + 10^{-6}M zeatin + 10^{-7}M NAA, on flower development during 14 days.

	nitrogen forms					
	control (BM)	KNO_3	NH_4NO_3	urea	$(NH_4)_2SO_4$	LSD (5%)
bud length (mm)	7.40	5.84	5.42	3.49	2.91	0.51
length of gynophore + pistil (mm)	4.36	3.10	3.26	1.11	0.77	0.78

levels is less pronounced with pistil development than with petal development, the 3% sucrose in the basal medium being adequate for the former. The osmotic value of the medium has little effect, as is shown when mannitol is added to make the medium equimolar to 6% sucrose; the mannitol itself turned out to be slightly inhibitory to the development of the flower.

Effect of nitrogen compounds. In order to further refine the medium the effect of varying levels of organic nitrogen compounds was studied (Table 3); however, none of the additions tested improved the growth of the flower bud. There was no difference in the effect of filter-sterilized or autoclaved glutamine.

Complete replacement of the nitrogen in the BM by urea inhibits the growth of the flower bud (Table 4). Also according to the effect of $(NH_4)_2SO_4$, nitrate is essential for bud development, a combination of nitrate with ammonia as present in the BM being the most favourable.

To analyse this further, the levels of ammonium and nitrate were varied by replacing the nitrogen in the BM by $NaNO_3$ and NH_4Cl or $(NH_4)_2$-succinate, and replenishing potassium with KCl (Fig. 5). Nitrate is required for flower bud growth, the 40 meq/l in the BM are about adequate, higher concentrations being inhibitory. Pistil development requires less nitrate than petal development. The preference of NH_4Cl over $(NH_4)_2$-succinate may be due to better uptake of the former.

Similar experiments for ammonium showed that ammonium is not essential, but further stimulates flower development at low concentrations, whereas higher amounts are toxic. The interaction between nitrate and ammonium was studied by adding $NaNO_3$ and NH_4Cl to the nitrogen-free BM (Fig. 6). The interaction is highly significant, both for bud and for pistil length, the benificial effect of low amounts of ammonia being apparent at the higher nitrate levels only. The best combination turned out to be 20 meq NO_3/l + 5 meq NH_4/l.

Effects of other nutrients. Potassium is present in the BM as KNO_3 and KH_2PO_4. Without potassium no growth occurred. A slight amount of potassium was already sufficient for the development of both petals and pistil, higher amounts did not impede growth.

The effect of phosphate was measured in a concentration range of KH_2PO_4. Without phosphate growth was absent, half the amount present in the BM gave optimum development already. The effect of calcium was

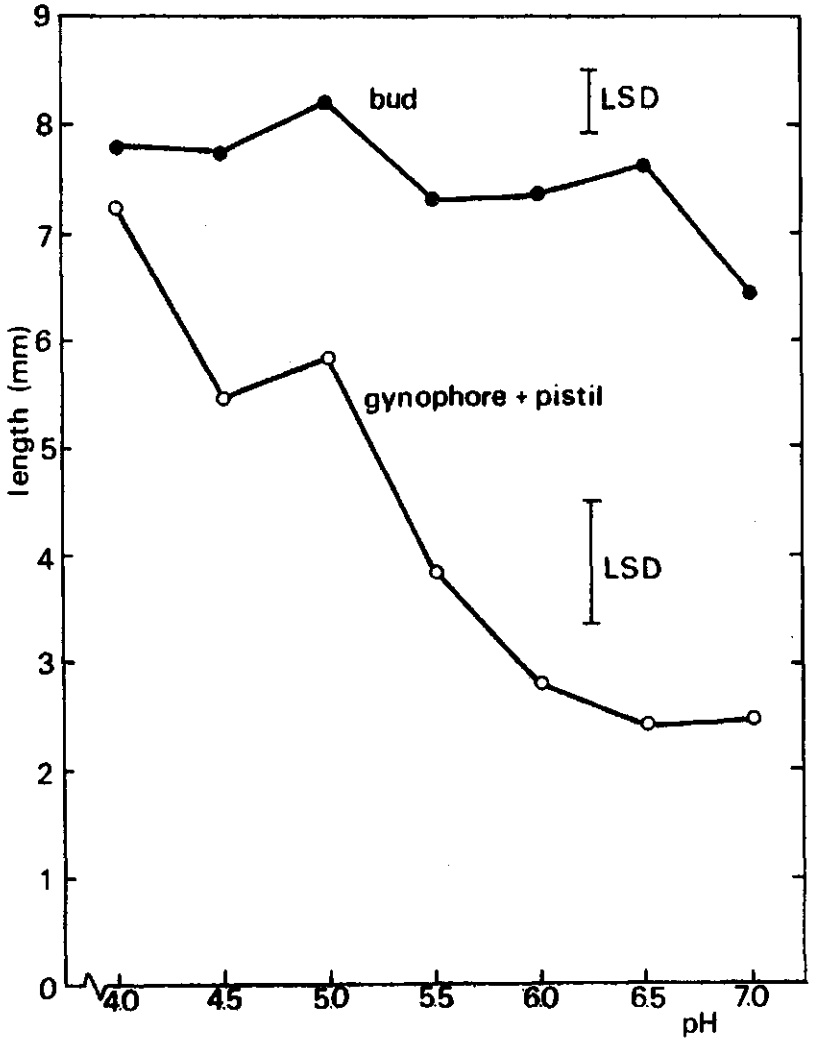

Fig. 3:

Effect of pH on the development of flower buds grown on BM + 10^{-6}M zeatin + 10^{-7}M NAA during 14 days.

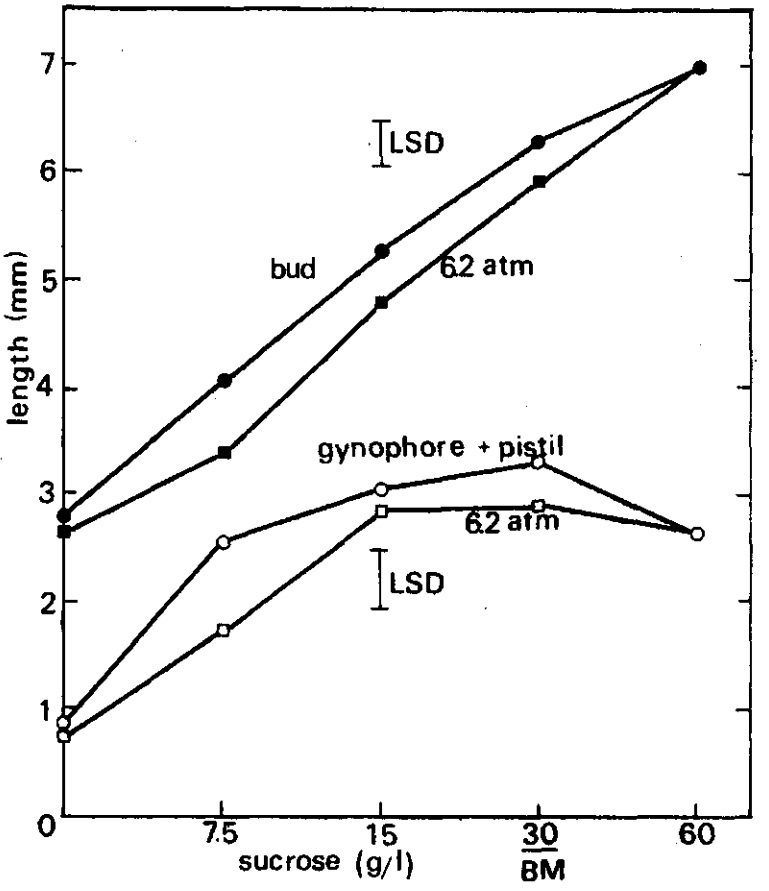

Fig. 4:

Effects of sucrose and mannitol on the development of flower buds grown on BM + 10^{-6}M zeatin + 10^{-7}M NAA during 14 days. The osmotic value of 6.2 atm (BM + 60 g sucrose/l) was obtained by addition of mannitol.

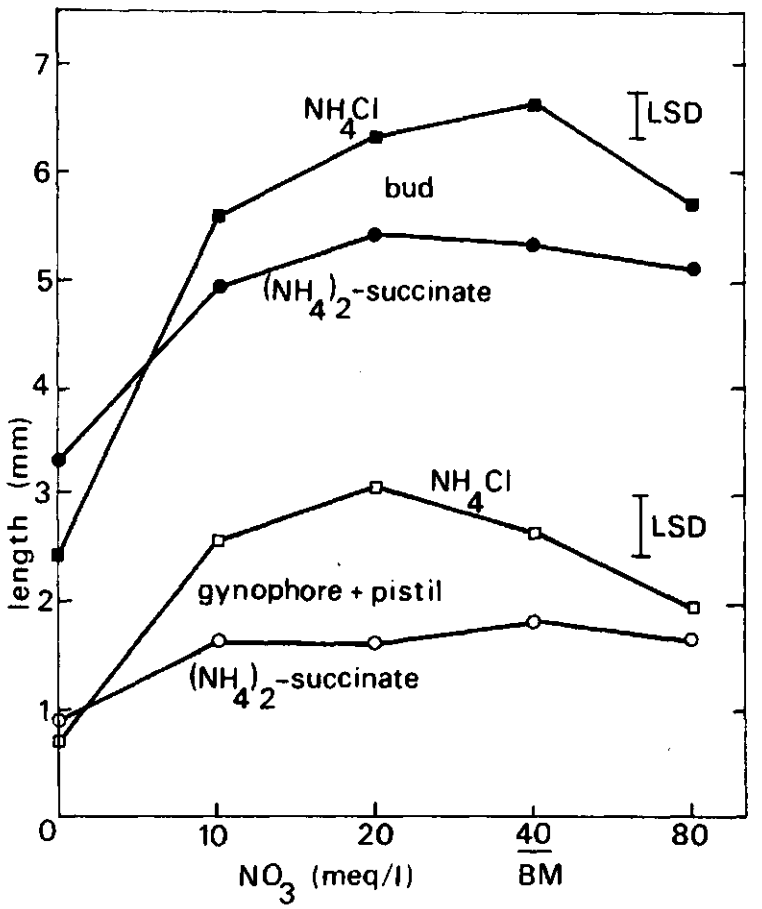

Fig. 5:

Effect of nitrate on the development of flower buds grown on nitrate-free BM + 10^{-6}M zeatin + 10^{-7}M NAA for 14 days. Different forms of ammonium added in equimolar amounts as present in BM.

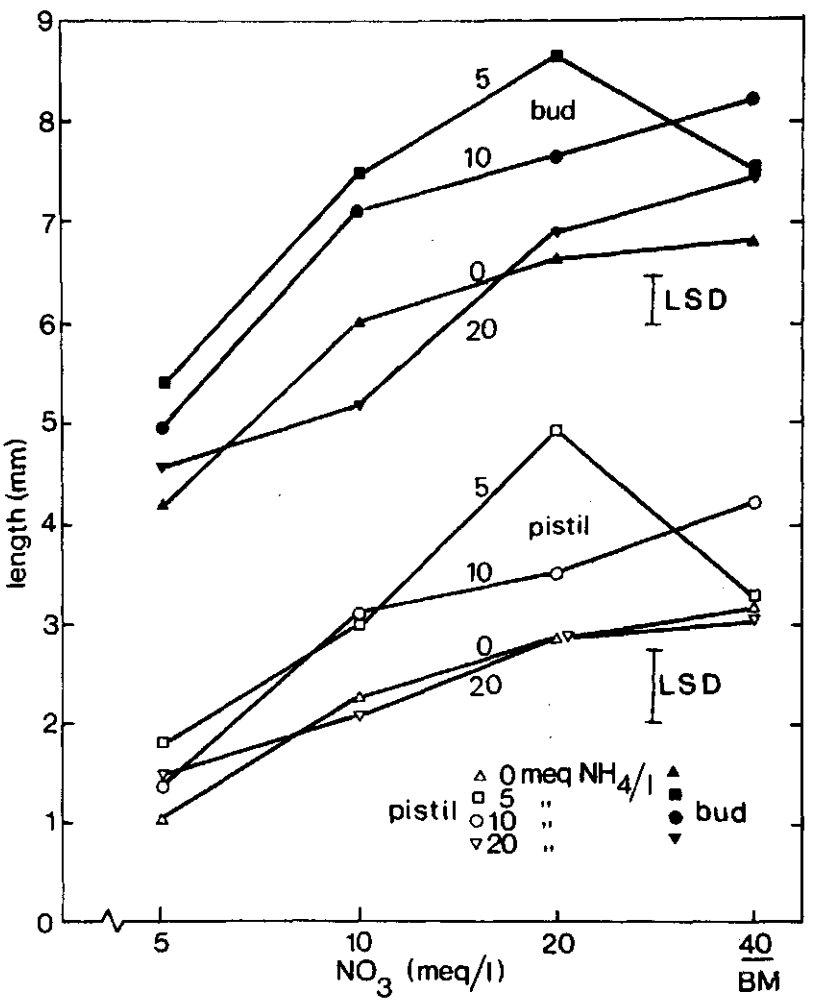

Fig. 6:

Effect of the interaction between nitrate and ammonium on the development of flower buds grown on nitrogen-free BM + 10^{-6}M zeatin + 10^{-7}M NAA for 14 days. Nitrate and ammonium are added respectively as $NaNO_3$ and NH_4Cl.

hardly significant.

Interactions of nutritive and hormonal factors. The interactions of sucrose and of nitrogen sources with several growth-regulating substances were analysed. However, the only significant interaction found was that between sucrose and cytokinins. As an example, Fig. 7 presents the interaction of sucrose and zeatin, the zeatin effect on bud and pistil growth being more pronounced at the lower than at the higher sucrose levels. Again petal elongation turned out to be far more dependent on the sucrose level than pistil growth (cf. Fig. 4).

This growth of the pistil is mainly sensitive to the levels of nitrate and ammonium salts and of a suitable cytokinin, e.g. zeatin. It can be concluded from Fig. 8 that the optimum conditions are attained at 20 meq NO_3, 5 meq NH_4, and 10^{-6}M zeatin. The effects of growth-regulating substances on pistil growth in vitro are further analysed by de Jong and Bruinsma (1974b).

DISCUSSION

The experiments demonstrate that Cleome iberidella flower buds of about 2.5 mm length can be successfully grown in vitro cultures to perfect flowers of about 8 mm length in 14 days. When the buds were allowed to develop further, they attained at the size of flower buds in vivo in another week, but often their organs abscised before opening.

The different effect of the acidity of the medium on the growth of petals and pistils needs further investigation, the favourable pistil growth at lower pH-values might have interesting prospects for floral organ culture in vitro.

The analysis of sucrose and zeatin interaction indicates that the best growth is obtained at 3% sucrose, as present in the BM, enriched with 10^{-6}M zeatin.

The study on nitrogen nutrition reveals that nitrate is the primary source, organic nitrogenous substances other than those already present in the BM being not required. The effects of these components of the BM have not been tested. Galun et al. (1962) and Rodrigues Pereira (1968) found weak growth of cucurbit flowers on modified White medium, which may well be ascribed to nitrogen deficiencies in this medium. Only in the presence of nitrate a

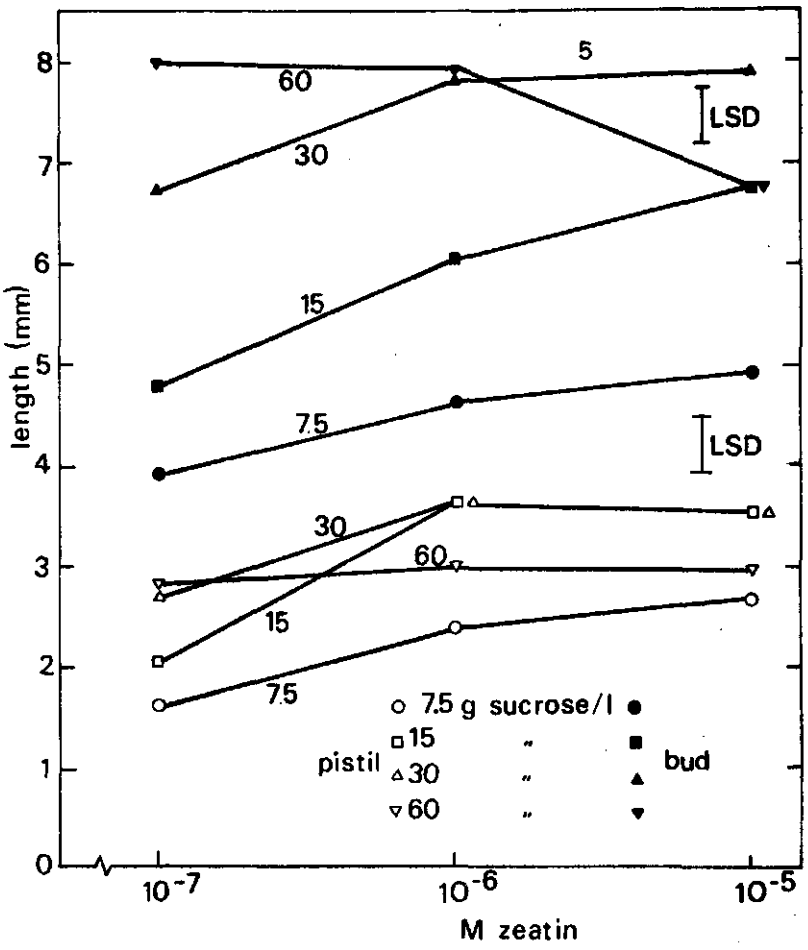

Fig. 7:
Effect of the interaction between sucrose and zeatin on the development of flower buds grown on BM + 10^{-7}M NAA for 14 days.

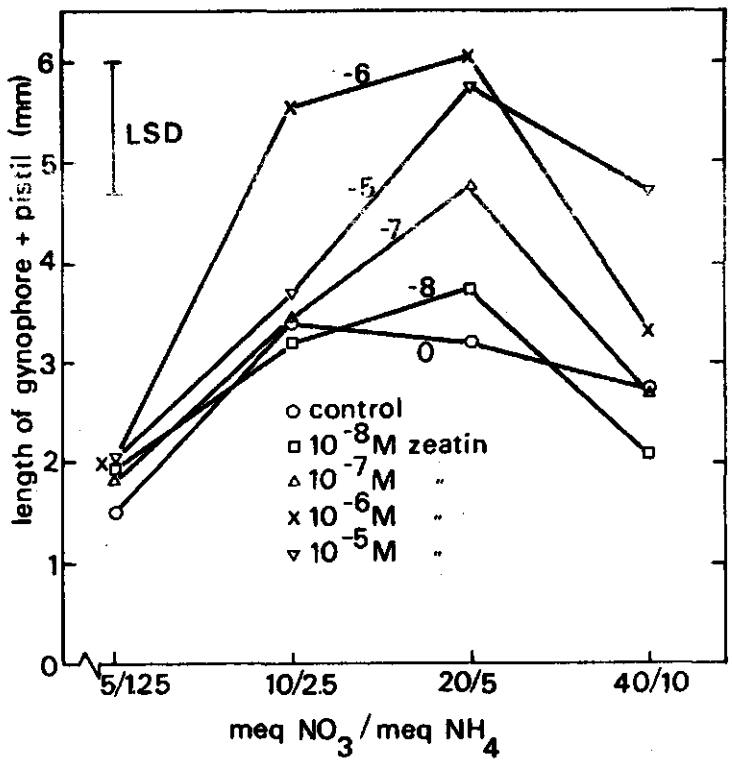

Fig. 8:
Effect of the interaction between zeatin and different nitrogen levels on the development of flower buds grown on nitrogen-free BM + 10^{-7}M NAA for 14 days. Nitrate and ammonium are added respectively as $NaNO_3$ and NH_4Cl.

further stimulating effect of low ammonium concentrations is found. This may be due to a rapid resumption of growth after inoculation by easy uptake of ammonium (Ferguson and Bollard, 1969) or, alternatively, ammonium might be directly used in the synthesis of nucleic acids (Jones et al., 1973). The stimulation by nitrate and the inhibiting effect of higher ammonium levels on pistil growth is also reported by Peterson (1973). In the final medium the nitrate level is halved, at 20 meq/l, and the ammonium is reduced to a fourth, at 5 meq/l.

A highly interesting outcome of the present study is the difference in nutrient requirement between petals, on the one hand, and gynophore and pistil, on the other hand. Figures 4, 5 and 7 show that at decreasing concentrations of carbohydrate and nitrogen bud growth becomes already inhibited, whereas the pistil development is still optimum. If the same nutrient dependency holds for Cleome spinosa flowers grown in vivo, the inhibition of pistil growth resulting in female sterility as found with these flowers in the field (de Jong and Bruinsma, 1974a) can hardly be ascribed to nutrient deficiency. For lack of nutritive factors is unlikely then to inhibit the development of the pistil to a larger extent than that of the petals. Hence, development of male instead of perfect flowers, due to competition between developing fruits and flower buds at the inflorescence (de Jong and Bruinsma, 1974a), is rather to be ascribed to hormonal than to nutritive factors. Improved nutritive conditions may change hormone levels, for instance by stimulating cytokinin biosynthesis (Phillips, 1968) and, therebye, favour pistil development which, according to the present study, requires such growth-regulating substances. This is further analysed by de Jong and Bruinsma (1974b and c).

ACKNOWLEDGEMENTS

The authors are most grateful to Mrs.Dr. C. Nitsch for allowing one of us (A.W.d.J.) te become acquainted with the techniques of culturing plant organs in vitro at the Laboratoire de la Physiologie Pluricellulaire du Centre National de la Recherche Scientifique, at Gif-sur-Yvette, France. We wish to thank Mrs. J.H. IJff for her

technical assistance. This investigation was supported by the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

REFERENCES

Ferguson, A.R. and E.G. Bollard: Nitrogen metabolism of Spirodela oligorrhiza. I. Utilization of ammonium, nitrite and nitrate. Planta 88: 344-352 (1969).

Galun, E., A. Yung and A. Lang: Culture and sex modification of male cucumber buds in vitro. Nature 194: 596-598 (1962).

Jones, L.H., J.N. Barrett and P.P.S. Gopal: Growth and nutrition of a suspension culture of Pogostemon cablin Benth. (Patchouli). J.Exp.Bot. 24: 145-158 (1973).

Jong, A.W. de and J. Bruinsma: Pistil development in Cleome flowers. I. Effects of fertilization and of the presence of leaves and fruits on female abortion in Cleome spinosa Jacq. Z.Pflanzenphysiol. : - (1974a).

- - : Pistil development in Cleome flowers. III. Effects of growth-regulating substances on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro. Z.Pflanzenphysiol. : - (1974b).

- - : Pistil development in Cleome flowers. IV. Effects of growth-regulating substances on female abortion in Cleome spinosa Jacq. Z.Pflanzenphysiol.. : - (1974c).

Peterson, C.M.: Nutritional requirements for ovule formation in excised pistils of Nigella. Amer.J.Bot. 60: 381-386 (1973).

Phillips, I.D.J.: Nitrogen, phosphorus and potassium distribution in relation to apical dominance in dwarf bean (Phaseolus vulgaris c.v. Canadian Wonder). J.Exp.Bot. 19: 617-627 (1968).

Rodrigues Pereira, A.S.: Sex expression in floral buds of Acorn squash. Planta 80: 349-358 (1968).

Ir. A.W. de Jong, A.L. Smit and Prof.Dr. J. Bruinsma,
Department of Plant Physiology, Agricultural University,
Arboretumlaan 4, Wageningen, The Netherlands.

PISTIL DEVELOPMENT IN CLEOME FLOWERS. III. EFFECTS OF GROWTH-REGULATING SUBSTANCES ON FLOWER BUDS OF CLEOME IBERIDELLA WELW. EX OLIV. GROWN IN VITRO

A.W. de Jong and J. Bruinsma

Department of Plant Physiology,
Agricultural University, Wageningen

Offered for publication in
Zeitschrift für Pflanzenphysiologie

Department of Plant Physiology, Agricultural University, Wageningen

Pistil development in Cleome flowers. III. Effects of growth-regulating substances on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro.

A.W. de Jong and J. Bruinsma

With 8 figures

SUMMARY

Pistil development in flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro is strongly stimulated by specific cytokinins, viz. zeatin and BA; kinetin is less active and 2iP ineffective. In the presence of zeatin, low concentrations of NAA stimulate pistil development, whereas higher concentrations are inhibitory.

Gibberellins cause pistil abortion, this effect is counteracted by ABA, Ethephon, and Chlormequat. In the absence of gibberellin, these substances and other growth retardants reduce pistil growth to a greater or less extent, ABA, being particularly inhibitory. A mechanism concerning the regulation of sexual expression in Cleome is discussed.

INTRODUCTION

Pistil development in Cleome iberidella flower buds grown in vitro remains optimum at decreasing nutrient concentrations which limit petal growth already (De Jong et al., 1974). Therefore, stimulation and repression of pistil development are to be ascribed to hormonal rather than to nutritive factors. For this reason, the effects of growth-regulating substances on flower bud and pistil growth were analysed.

MATERIAL AND METHODS

The techniques and the basal medium (BM) used were described by De Jong et al. (1974). They attained to a new basal medium (NBM) in which the nitrate level was halved, and ammonium was reduced to a fourth: KNO_3 (2000 mg/l) and NH_4NO_3 (1500 mg/l) were replaced by KCl (740 mg/l), $NaNO_3$ (1640 mg/l) and NH_4Cl (200 mg/l). On NBM, flower buds were grown during 9 days instead of 14 days because of the increase in growth rate. The sterilization of flower buds was also modified. Buds were sterilized in 200 ml 0.15% NaOCl and 0.005% Triton X-100 for 5 min. Thermolabile compounds were added to the media after sterilization through a Millipore filter, pore size 0.45 µm.

Abbreviations: ABA, abscisic acid; BA, benzylaminopurine; Chlormequat, (2-chloroethyl)trimethylammonium chloride; Ethephon (2-chloroethyl)-phosphonic acid; GA, gibberellin, 2iP, isopentenylaminopurine (γ,γ-dimethylallylaminopurine); NAA, naphtaleneacetic acid.

RESULTS

Without the addition of growth-regulating substances, the growth of the flower buds is rather good, but the growth of the pistil is very poor, yielding predominantly male flowers. Therefore, the effect of filter-sterilized milk from young coconuts, being a source of growth-regulating compounds, was studied. However, also because of the differences in effectivity of different samples, the further use of coconut milk was rejected, and the effects of pure regulating substances were analysed.

Effects of cytokinins. According to Fig. 1 bud development is hardly influenced by cytokinins, but pistil growth is stimulated by zeatin and BA, and, to a smaller extent, by kinetin. The natural cytokinin, 2iP, has no promoting effect on pistil growth. Adenine was tested in the range from 10^{-8} to 10^{-4}M, but turned out to be ineffective. As a result of higher BA concentrations, the base of the pistil swelled. When added to BM, kinetin had no significant effect, but zeatin and BA again considerably promoted pistil growth. As a consequence they strongly reduced female abortion (see e.g. Fig. 3).

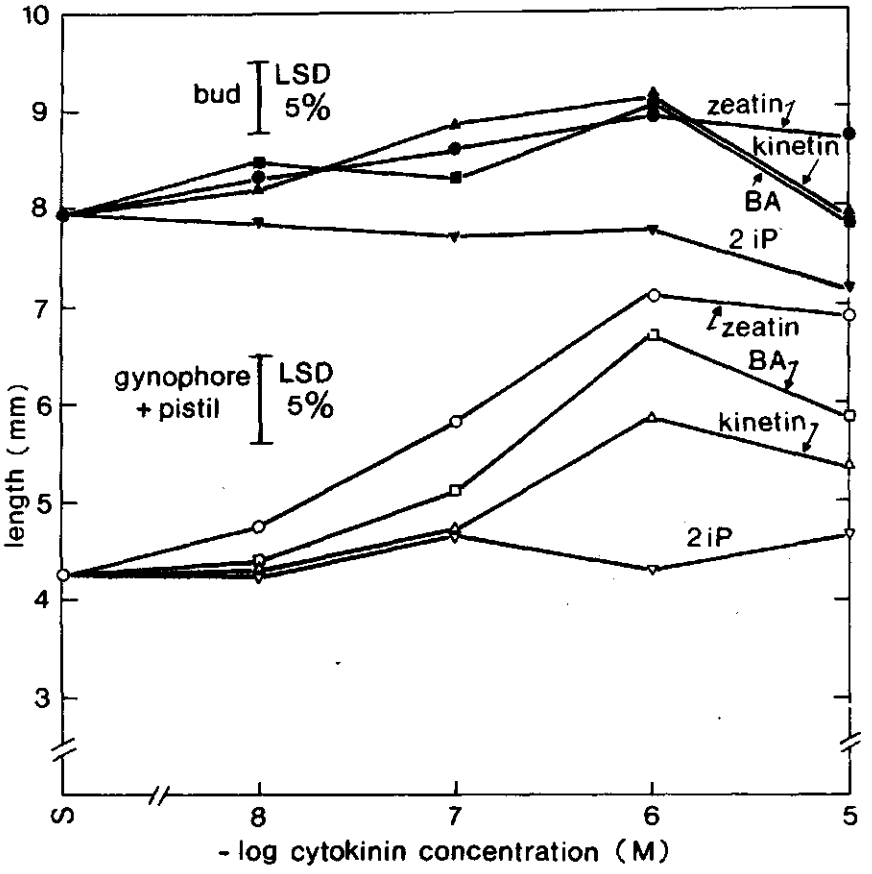

Fig. 1:
Effects of different cytokinins on the development of flower buds grown on NBM for 9 days.

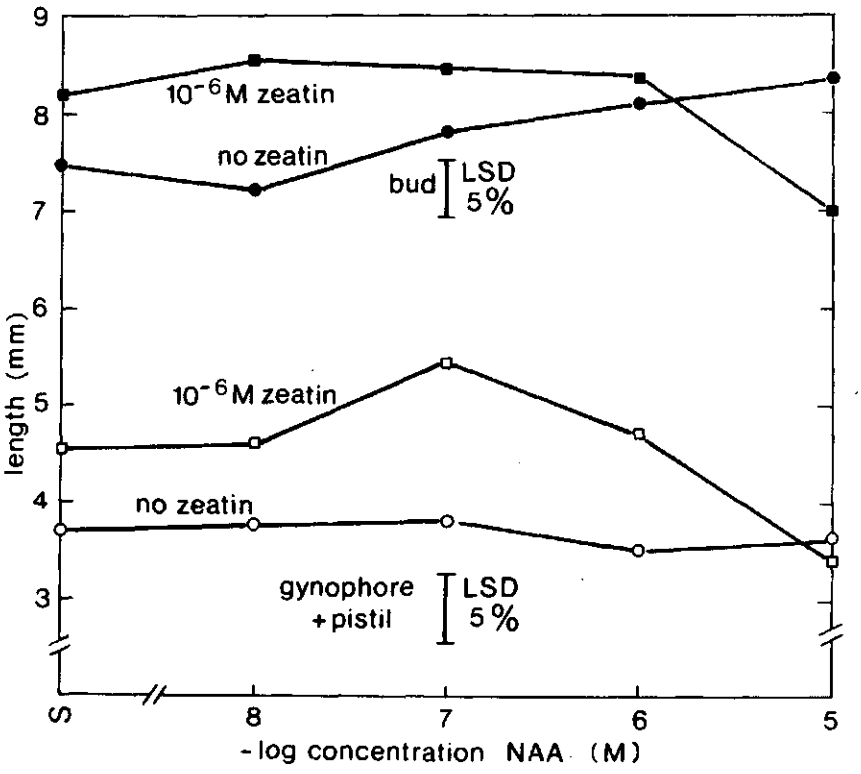

Fig. 2:
Interaction between zeatin and NAA in the development of flower buds grown on NBM for 9 days.

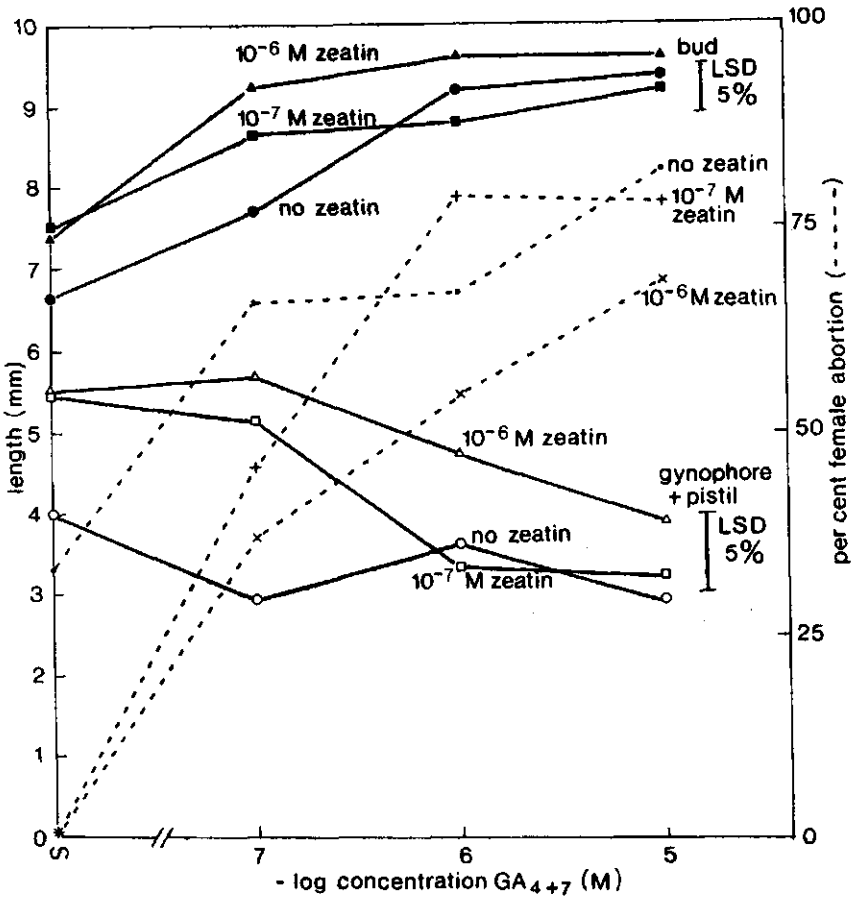

Fig. 3:
Interactions between zeatin and GA_{4+7} in the development of flower buds grown on NBM for 9 days.

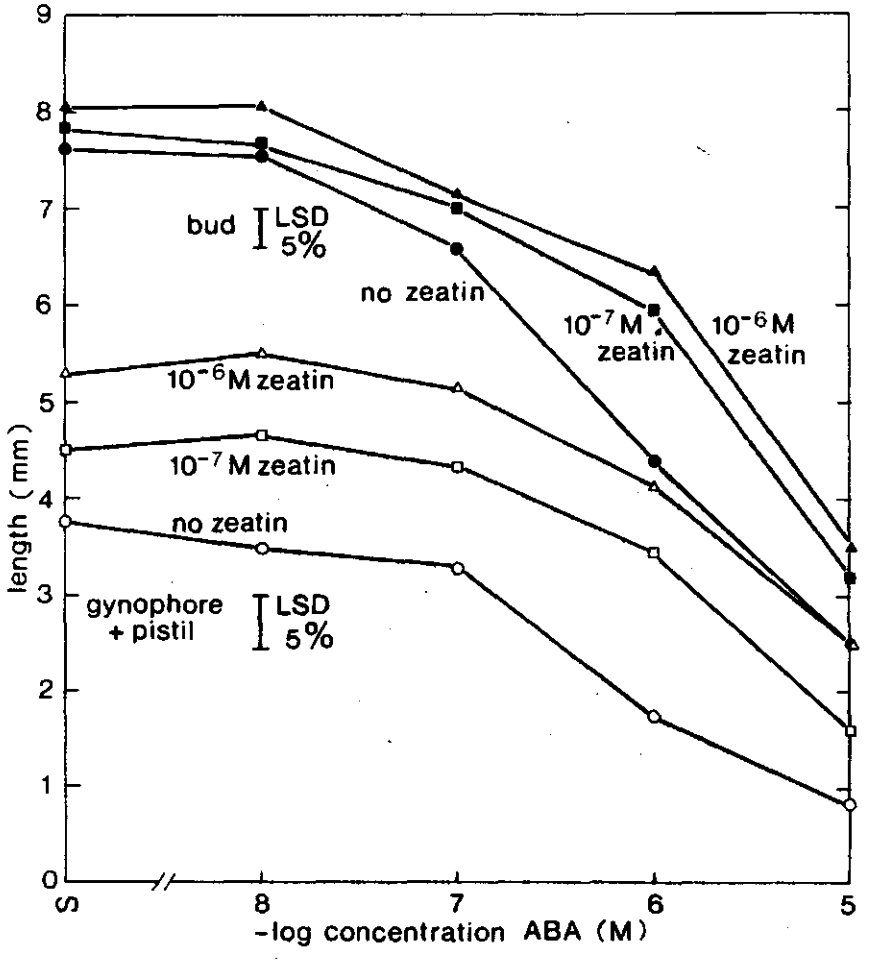

Fig. 4:
Interaction between zeatin and ABA in the development of flower buds grown on NBM for 9 days.

Effect of auxins. Fig. 2 represents the interaction between NAA and zeatin in flower development. Without zeatin, NAA promotes petal growth, but when zeatin is added to the medium this positive NAA effect no longer occurs, 10^{-5}M NAA being even inhibitory. Without zeatin, there is no effect of NAA on pistil growth, but in the presence of 10^{-6}M zeatin low amounts of NAA (10^{-7}M and occasionally also 10^{-8}M) are stimulatory, and high amounts (10^{-5}M) invariably inhibitory, to pistil growth.

The action of such other auxins as indoleacetic acid (IAA, filter-sterilized), indolebutyric acid (IBA), and 2,4-dichlorophenoxyacetic acid (2,4-D), generally resembles that of NAA. The anti-auxin, 2,3,5-triiodobenzoic acid (TIBA), has no regulating effect but is very toxic at higher concentrations. Filter-sterilized tryptophan decreases petal growth at 10^{-4}M, but has no effect on pistil development.

Effect of gibberellins. GA_{4+7} promotes bud growth and inhibits pistil development (Fig. 3). Without zeatin the effect of GA_{4+7} on pistil development is not distinct, but the zeatin-induced increase of pistil development is reduced by GA_{4+7}. The effect of GA_{4+7} on pistil growth is two-sided: on the one hand, it causes female abortion, antagonizing zeatin, but, on the other hand, the few pistils that escape this aborting effect are rather stimulated in growth, causing in many trials a confusing of the results. The effect of GA_3 resembles that of GA_{4+7} as far as pistil development and female abortion are concerned, but petal growth is not promoted by GA_3.

Effect of ABA. The growth of both petals and pistils is inhibited by ABA (Fig. 4), practically no growth occurring at 10^{-5}M ABA. There is no interaction with zeatin in bud or pistil growth. NAA, like zeatin, diminishes the sensitivity of pistil development to ABA, resulting in a shift towards higher ABA concentrations required for pistil growth inhibition.

ABA, unlike gibberellins, reduces pistil growth without completely blocking its development. On the contrary, ABA even removes the abortive effect of gibberellins in pistil development (Fig. 5). Both ABA and GA_{4+7}, when added, separately, inhibit

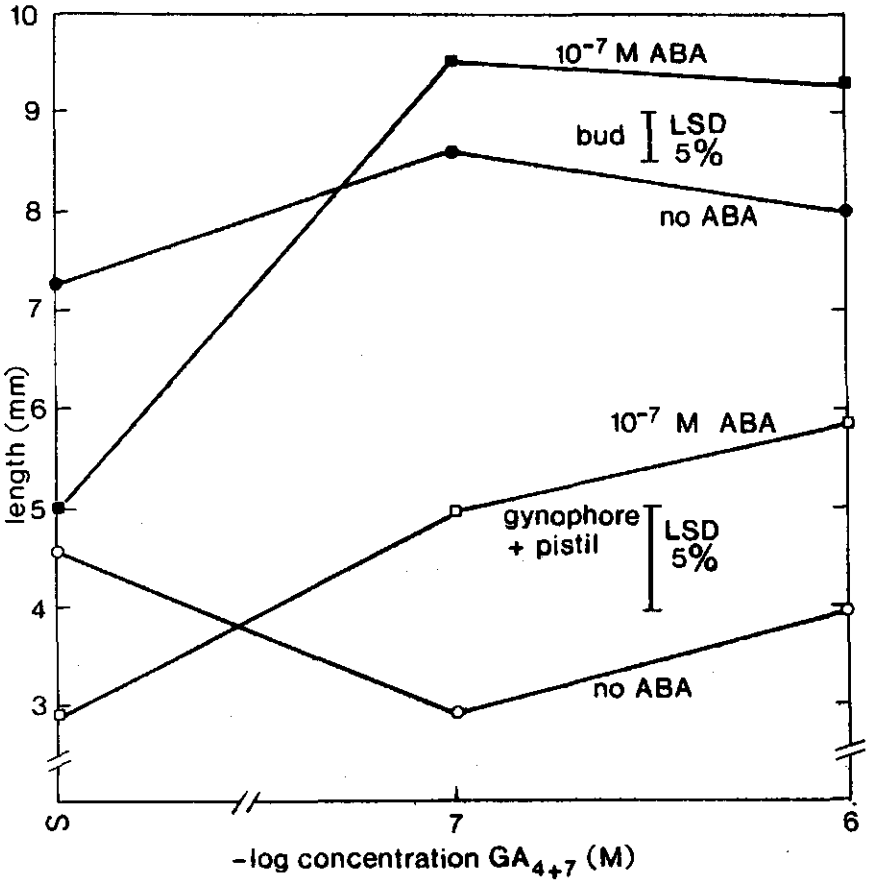

Fig. 5:
Interaction between ABA and GA_{4+7} in the development of flower buds grown on NBM + 10^{-7}M zeatin for 9 days.

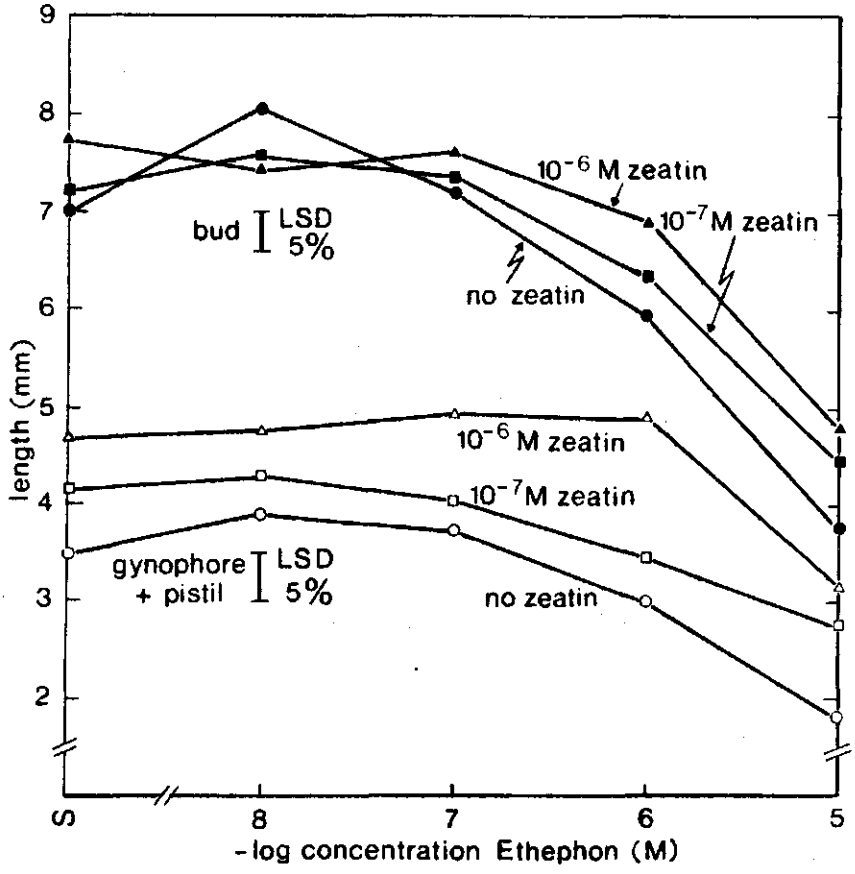

Fig. 6:
Interaction between Ethephon and zeatin in the development of flower buds grown on NBM for 9 days.

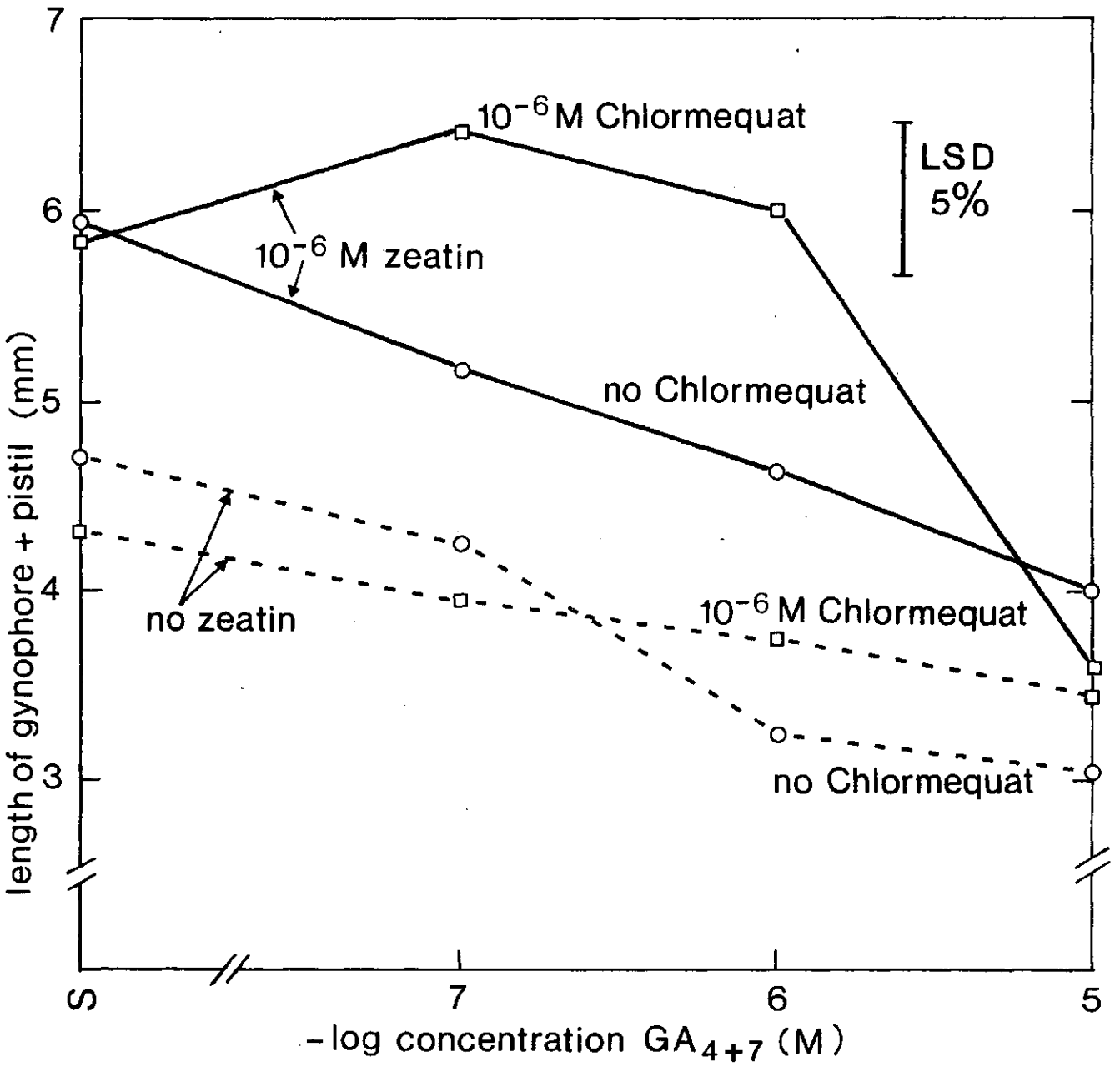

Fig. 7:
Interaction between zeatin, Chlormequat, and GA_{4+7} in the pistil development of flower buds grown on NBM for 9 days.

pistil growth reduction, the latter by its aborting effect. Added simultaneously, they counteract their mutual effects, ABA by reducing the gibberellin-induced abortion, GA_{4+7} by removing the growth inhibition exerted by ABA.

Effect of Ethephon. The effect of the ethylene-releasing substance, Ethephon, at different zeatin levels in NBM, is shown in Fig. 6. The ethylene concentration in the atmosphere over a medium containing 10^{-6}M Ethephon was about 1 ppm. Ethephon inhibits petal and pistil growth at higher concentrations, 10^{-6}M zeatin reduces this effect, particularly with pistil growth. There is no influence of Ethephon on female abortion. Ethephon counteracts the effect of gibberellins and vice versa, in the same manner as ABA, although not always significantly.

Effect of growth retardants. The growth retardant, Chlormequat, SADH (succinic acid-2,2-dimethylhydrazide), and ACPC (2-isopropyl-4-trimethylammoniumchloride-5-methylphenylpiperidine-1-carboxylate), hardly affect petal and pistil growth, CBBP (2,4-dichlorobenzyl-tributylphosphonium chloride) being inhibitory at higher concentrations.

Without zeatin there is no significant interaction between GA_{4+7} and Chlormequat on pistil development (Fig. 7). However, when 10^{-6}M zeatin is added to the medium, the inhibiting effect of GA_{4+7} is removed by 10^{-6}M Chlormequat, except at the highest gibberellin concentration. Chlormequat slightly reduces the GA_{4+7}-induced female abortion, the pistils escaping this aborting effect being of the same size with and without Chlormequat, indicating that Chlormequat, too, counteracts the pistil-aborting effect of GA_{4+7} rather than affecting pistil growth.

DISCUSSION

Without the addition of growth regulators, predominantly male flowers develop from buds grown in vitro. The cytokinins zeatin, BA and, to a lesser extent, kinetin, are the only growth-regulating substances which considerably enhance pistil development, while 2iP is inactive (Fig. 1). Total bud growth may be somewhat stimulated, but to a much smaller extent, so that the cytokinins, zeatin and BA,

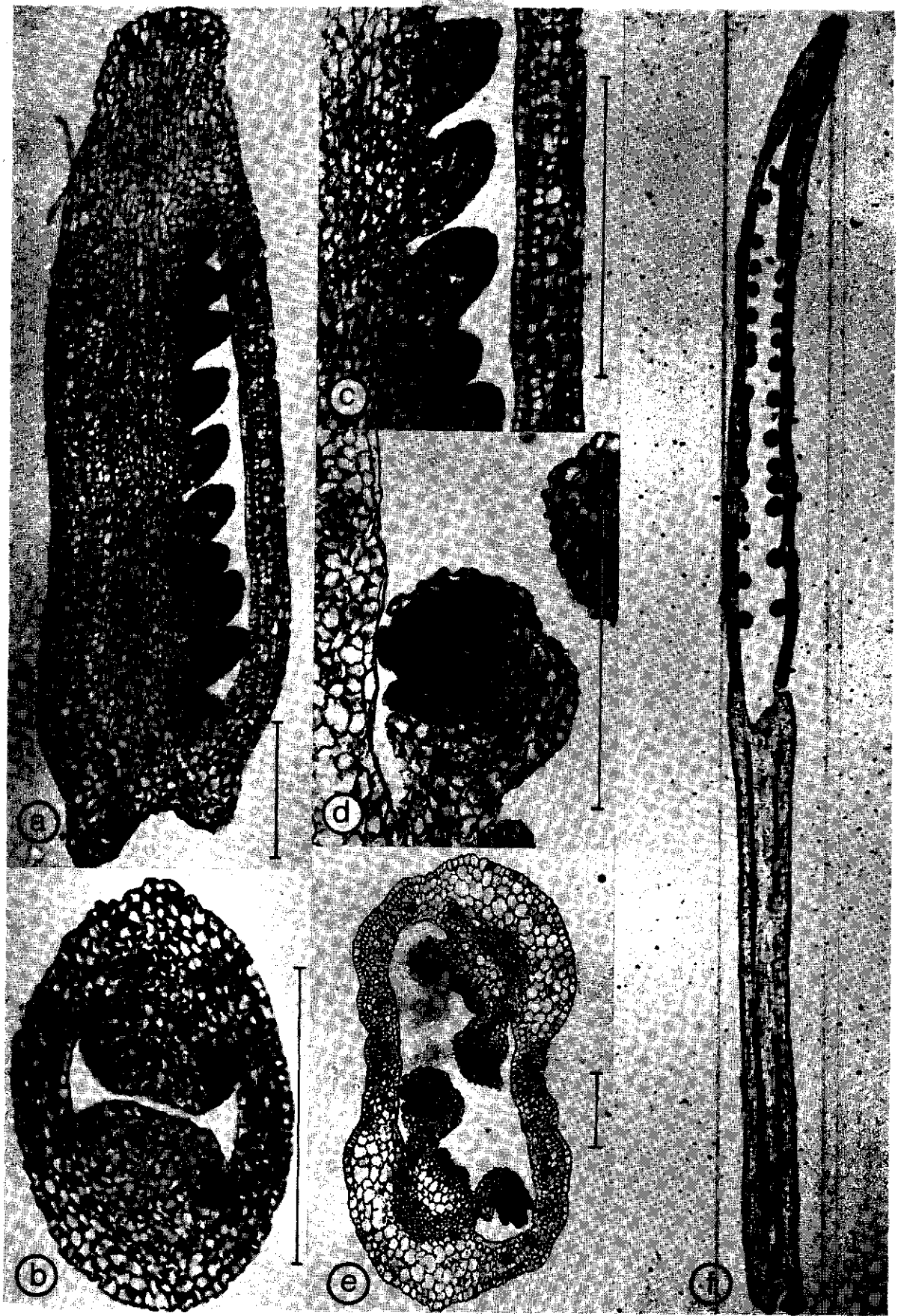

Fig. 8:

Pistil development at the beginning and the end of the culture in vitro. a. longitudinal section at the time of inoculation. b. cross section at the time of inoculation. c. detail of a. d. detail of e. e. cross section nine days after inoculation. f. longitudinal section nine days after inoculation.

enable the buds to develop into perfect bisexual flowers. These cytokinins are required for the final cell divisions in the pistil. Microscopic studies demonstrated that at the stage of inoculation of the flower buds on the medium the ovules are still differentiating (Fig. 8). A similar effect of cytokinins on pistil development was found by Negi and Olmo (1972) with Vitis vinifera, where cytokinin accelerated mitotic divisions in pistillate tissue.

The results demonstrate that effects of growth-regulating substances on pistil development can only be studied when e.g. zeatin is present in the medium. They fail to show up when the cytokinin level is limiting (Figs. 2, 3, 6 and 7). In the presence of zeatin, NAA (Fig. 2) and GA_{4+7} (Fig. 3) affect pistil growth. NAA significantly stimulates at lower, and inhibits at higher concentrations, whereas GA_{4+7} invariably inhibits pistil development. The abortive effects of these growth regulators, found in experiments with intact plants of Cleome spinosa (De Jong and Bruinsma, 1974b) can thus be partly reproduced in the in vitro experiments with Cleome iberidella. The gibberellin effect can be ascribed to a direct influence on pistil development, that of auxin may be more complicated.

Contrary to the expectations on the ground of data in the literature of sexual expression (e.g. Rudich et al., 1969; Mohan Ram and Jaiswal, 1970), it was found that Ethephon (Fig. 6) decreases pistil growth, although this effect was reduced when zeatin was added to the medium. The inhibition of pistil development by GA_{4+7}, leading to abortion, was counteracted by ABA, Ethephon, and Chlormequat, although this last substance hardly affected pistil growth of its own. This implies that also Chlormequat does not interfere with the synthesis of gibberellins but rather with their action in female abortion. Chlormequat overcomes, up to equimolar amounts, the gibberellin-induced aborting effect, but, like ABA and Ethephon, fails to reduce the gibberellin-induced growth stimulation of those pistils which escape the aborting effect. Increased feminization by Chlormequat was also found with cucurbitaceous plants in vivo (Mishra and Pradhan, 1970; Gosh and Bose, 1970; Saimbi and Thakur, 1973). Unlike our results, in these and other cases there were no indications that the action of Chlormequat was not mediated via an effect on the

endogenous gibberellin synthesis.

The reversal of the effect of GA_{4+7} on pistil development by ABA fits in with the observation by Mohan Ram and Jaiswal (1972) of an inhibition by ABA of gibberellin-induced male flowering on female Cannabis plants.

The analysis of the effects of growth-regulating substances and their interactions in vitro, allows for a hypothesis describing the mechanism underlying the regulation of sexual expression in Cleome species. The development of the pistil requires specific cytokinins, e.g. zeatin, for the final cell divisions, which are completed earlier already in the other flower parts. Because in vivo a cytokinin supply from the roots occurs, this requirement only shows up when flower buds are grown in vitro on defined media. Cytokinins, possibly together with low amounts of auxins, allow for the completion of flower formation. This development can be directly inhibited by gibberellins causing the pistil to abort. De Jong and Bruinsma (1974a) demonstrated that pistil growth is stimulated by the removal of young leaves and of developing seeds, which are possible sites of gibberellin production.

Developing fruits, causing female abortion in vivo, may affect pistil development in flower buds superior at the inflorescence in two ways: by producing gibberellin-like hormones directly inhibiting pistil development, and by limiting the supply of nutrients and cytokinins to the flower buds by their competitive sink action. The first possibility, however, is ruled out by the finding that the effect of developing fruits on pistil development is optimum already when their gibberellin content is still very low, rather coinciding with the peak in IAA content (De Jong and Bruinsma, 1974a). As for the second possibility, lack of mineral or organic nutrients reduces the growth of the petals rather than that of the pistil (De Jong et al., 1974), so that the abortive effect of fruits on pistil development in the superior flower buds at the inflorescence is likely to be mainly ascribed to competition for cytokinins derived from the roots. This is further analysed in experiments with intact plants (De Jong and Bruinsma, 1974b).

ACKNOWLEDGEMENTS

We wish to thank Mrs. J.H. IJff, Mr. J. Vos and Mr. A.P. Everaarts for their co-operation, and Mr. A.J. Munting for his help in microtechnical preparations. This investigation was partly supported by the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

REFERENCES

Gosh, M.C. and T.U. Bose: Sex modification in cucurbitaceous plants by using CCC. Phyton 27: 131-135 (1970).

Jong, A.W. de and J. Bruinsma: Pistil development in Cleome flowers. I. Effects of nutrients and of the presence of leaves and fruits on female abortion in Cleome spinosa Jacq. Z. Pflanzenphysiol. : - (1974a).

- - : Pistil development in Cleome flowers. IV. Effects of growth-regulating substances on female abortion in Cleome spinosa Jacq. Z. Pflanzenphysiol. : - (1974b).

Jong, A.W. de, A.L. Smit and J. Bruinsma: Pistil development in Cleome flowers. II. Effects of nutrients on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro. Z. Pflanzenphysiol. : - (1974).

Mishra, R.S. and B. Pradhan: The effect of (2-chloroethyl)-trimethylammoniumchloride on sex expression in cucumber. J. hort. Sci. 45: 29-31 (1970).

Mohan Ram, H.Y. and V.S. Jaiswal: Induction of female flowers on male plants of Cannabis sativa by Cepha. Experientia 26: 214-216 (1970).

- - : Induction of male flowers of Cannabis sativa by gibberellins and its inhibition by abscisic acid. Planta 105: 263-266 (1972).

Rudich, J., A.H. Halevy and N. Kedar: Increase in femaleness of three cucurbits by treatment with Ethrel, an ethylene releasing compound. Planta 86: 69-76 (1969).

Negi, S.S. and H.P. Olmo: Certain embryological and biochemical aspects of cytokinin SD 8339 in converting sex of male Vitis vinifera (sylvestris). Amer.J.Bot. 59: 851-857 (1972).

Saimbhi, M.S. and M.R. Thakur: Growth, sex expression and yield of squash melon (Citrullis vulgaris var. fistulosis) as influenced by 2(chloroethyl) phosphonic acid, 2,3,5--triiodobenzoic acid and (2-chloroethyl)trimethylammoniumchloride. Physiol. Plant. 28: 383-387 (1973).

Ir. A.W. de Jong and Prof.Dr. J. Bruinsma. Department of Plant Physiology, Agricultural University, Arboretumlaan 4, Wageningen, The Netherlands.

PISTIL DEVELOPMENT IN *CLEOME* FLOWERS. IV. EFFECTS OF GROWTH-REGULATING SUBSTANCES ON FEMALE ABORTION IN *CLEOME SPINOSA* JACQ.

A.W. de Jong and J. Bruinsma

Department of Plant Physiology,
Agricultural University, Wageningen

Offered for publication in
Zeitschrift für Pflanzenphysiologie

Department of Plant Physiology, Agricultural University,
Wageningen

Pistil development in Cleome flowers. IV. Effects of growth-regulating substances on female abortion in Cleome spinosa Jacq.

A.W. de Jong and J. Bruinsma

With 1 figure

SUMMARY

Pistil development in flower buds of andromonoecious Cleome spinosa Jacq. is inhibited by sprays with NAA and with gibberellins, and promoted by Ethephon and zeatin. The action of gibberellins and Ethephon is not exerted via the fruits, gibberellin directly causing pistil abortion, whereas Ethephon requires the presence of endogenous gibberellins.

Although exogenous auxin reduces pistil development, the main action of endogenous auxin in female abortion is rather to increase the sink activity of subjacent developing fruits, thus changing the competition for cytokinins from the roots. Hence, the intermittent formation of perfect and male flowers in zones on the inflorescence can be ascribed to a lack of cytokinins in flower buds on top of simultaneously developing fruits with a high sink activity.

INTRODUCTION

Pistil development in flower buds of Cleome spinosa Jacq. is inhibited by fruits developing underneath the buds at the inflorescence, and stimulated by mineral nutrition and the presence of mature leaves (De Jong and Bruinsma, 1974a). Pistil abortion in flower buds can be ascribed to the sink activity for nutritive and hormonal factors of these developing fruits, due to the auxin production by their seeds (De Jong and Bruinsma, 1974a, b). The in vitro culture of Cleome iberidella flower buds showed that pistil development, although being sensitive to nutritive deficiences, mainly depends on growth-regulating substances (De Jong et al., 1974; De Jong and Bruinsma, 1974b).

The effects of these substances on female abortion in Cleome spinosa plants were analysed by spraying the inflorescence or by local application to the fruit or stalk.

MATERIAL AND METHODS

Clones of cuttings of Cleome spinosa Jacq. and culture methods are described by De Jong and Bruinsma (1974a).

Solutions of ABA, GA_3, GA_{4+7}, kinetin and zeatin were made by dissolving the regulators in a few drops of 0.1 N KOH and dilution with distilled water. NAA was used as the potassium salt. Before application the pH was adjusted to 5.5, Triton X 100 (0.01%) was added as a wetting agent. Growth regulators were applied to the plants in different ways: a) by weekly spraying of the inflorescence with 10 ml per plant, starting at the onset of flowering; b) as a droplet (12 µl) to deseeded fruitlets seven days after pollination; c) by daily application with a syringe needle to the base of the flowering part of the inflorescence (zeatin only); d) Ethephon was applied according to b) or to the leaves only to avoid flower bud abscission.

The spectofluorometric determination of IAA was carried out according to Knegt and Bruinsma (1973).

Abbreviations: ABA, abscisic acid; Ethephon, (2-chloroethyl)phosphonic acid; GA, gibberellin; IAA, indoleacetic acid; NAA, naphtaleneacetic acid.

RESULTS

Tables 1 and 4 represent the effects of NAA, sprayed at weekly intervals during 9 weeks. The female abortion was considerably increased, probably by a direct inhibition of pistil growth because there was hardly any effect on the growth of the inflorescence, the number of flowers per day and the fruit growth. In other experiments the fresh and dry weights of seeds and carpels were also examined, showing no effect of NAA treatments. Application of NAA in deseeded fruitlets had no effect whatsoever.

Table 2 shows the effect of NAA spraying on the IAA content in fruits at different days after pollination. The optimum IAA level in fruits was achieved sooner in NAA-treated plants without, however, changing the ultimate fruit weight (Tables 1 and 4).

Table 1: Effect of NAA, applied to main inflorescences at seven-day intervals during 9 weeks, on pistil development of Cleome spinosa flowers. Eight replicates per treatment.

M NAA	0	3.10^{-6}	3.10^{-5}	3.10^{-4}	LSD (5%)
% female abortion	23.0	26.6	29.9	38.1	6.9
No. of flowers/day	3.7	3.6	3.7	3.5	n.s.[x)]
inflorescence length (cm)	50.4	53.6	49.1	49.4	4.4
fruit weight (g)	0.44	0.43	0.41	0.44	n.s.

x) n.s. = not significant

Table 2: Effect of 10^{-4}M solutions of NAA and gibberellins, sprayed at main inflorescences at weekly intervals, on the IAA content of developing fruits.

days after pollination	IAA content in ng per fruit control	NAA	GA_3	GA_{4+7}	LSD (5%)
10	680	780	600	470	85
16	840	680	890	320	85
30	280	200	230	230	45

Table 3: Effect of GA_3, applied to main inflorescences at seven-day intervals during 9 weeks, on pistil development of Cleome spinosa flowers. Seven replicates per treatment.

M GA_3	0	3.10^{-5}	10^{-4}	3.10^{-4}	LSD (5%)
% female abortion	21.2	27.8	24.3	25.5	5.6
No. of flowers per day	3.5	3.8	4.0	4.0	0.2
inflorescence length (cm)	51.3	49.7	54.0	58.6	6.9
fruit weight (g)	0.51	0.52	0.52	0.51	n.s.[x]

[x] n.s. = not significant

Table 4: Effect of 10^{-4}M solutions of NAA, GA_3 and GA_{4+7}, applied to main inflorescences at seven-day intervals during 9 weeks, on pistil deve pment of Cleome spinosa flowers. Ten replicates per treatment.

	control	NAA	GA_3	GA_{4+7}	LSD (5%)
% female abortion	21.9	30.4	28.5	29.3	5.0
inflorescence length (cm)	59.2	52.7	64.7	90.7	6.0
fruit weight (mg)	155	158	143	161	11
seed wt./fruit (mg)	40	43	35	22	6
No. of seeds/fruit	58	62	45	29	8

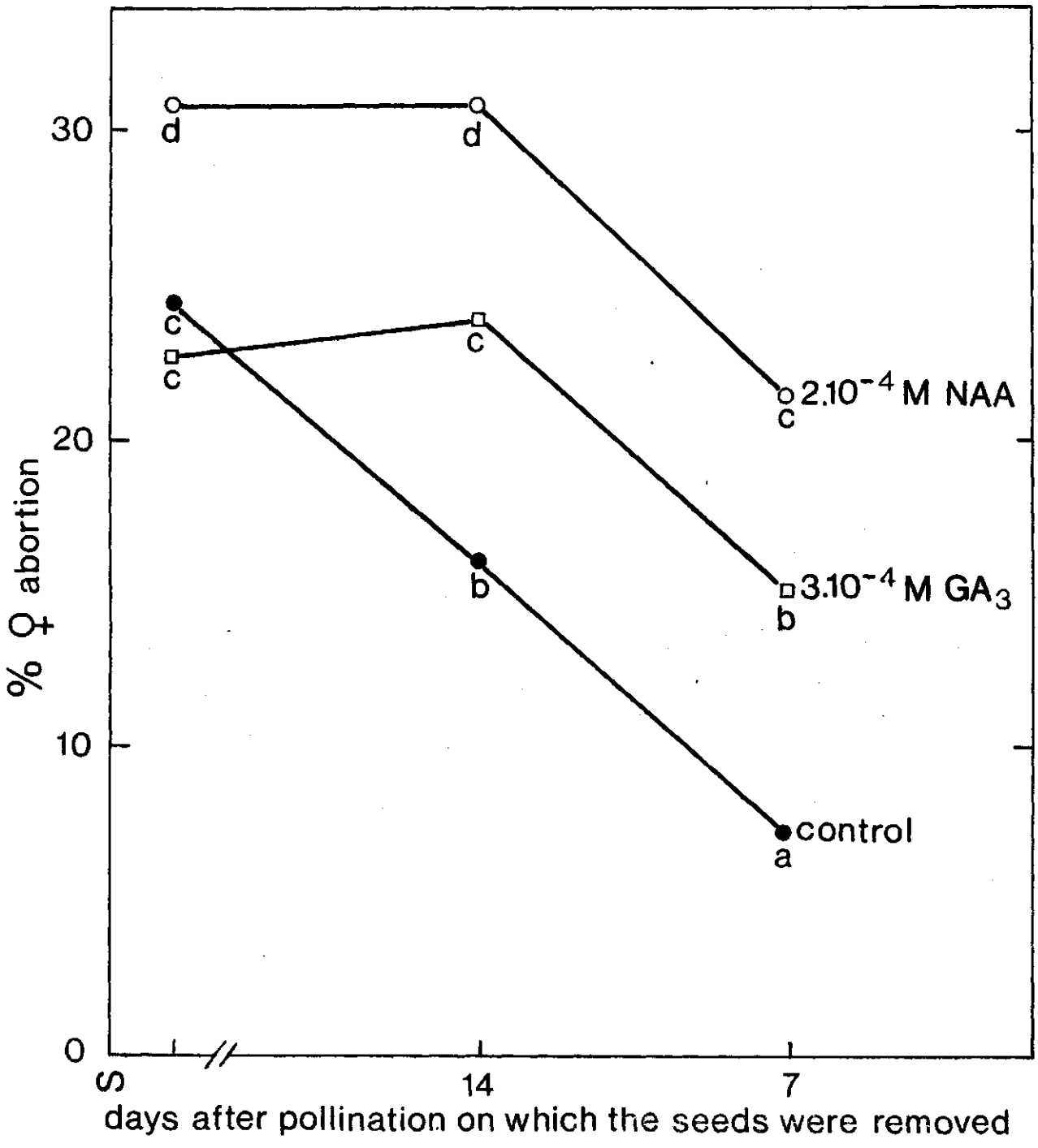

Fig. 1:
Effect of NAA and GA_3 applied at seven-day intervals during 8 weeks, on pistil development. Seed removed after 7 or 14 days. Nine replicates per treatment. LSD = 5.8% (5%).

To study the effect of gibberellins, plants were sprayed with GA_3 or GA_{4+7} (Tables 3 and 4). In most cases gibberellins increased female abortion, while the inflorescence and fruits of the treated plants were elongated; the fruits, however, showed considerable seed abortion. Therefore, and also to see whether sprayed regulators could replace the hormone-producing developing seeds, the effects of NAA and GA_3 on female abortion were also studied on plants with deseeded fruitlets (Fig. 1). When the seeds were removed 7 or 14 days after pollination, sprays of NAA and of GA_3 inhibited pistil development and counteracted the beneficial effect of seed removal on pistil growth; without seed removal the effect of GA_3 on pistil abortion did not show up.

Treatment with GA_{4+7}, being more effective than GA_3, caused a decrease of the IAA content in the fruits (Table 2), probably caused by the smaller amount of seeds in the fruits (see also Table 4).

Table 5 represents the effect of Ethephon on female abortion and fruit development. Ethephon caused a reduction of pistil abortion without affecting the growth of the inflorescence or the fruit development. Application of a drop of Ethephon solution in deseeded fruitlets also resulted in an increase of pistil development (Table 6); at higher concentrations this reduction of pistil abortion may be due to flower bud abscission and a decreased fruit set, but the beneficial effect on pistil development is also significant at 10^{-5}M Ethephon, where these factors do not yet interfere.

Kinetin and ABA, either sprayed (Table 7) or locally applied in deseeded fruitlets, had no effect on female abortion. Because zeatin stimulated pistil development in Cleome iberidella flower buds grown in vitro, while kinetin was rather ineffective (De Jong and Bruinsma, 1974b), zeatin was added daily to the base of the flowering part of the inflorescence with the aid of a syringe needle. Because it is generally accepted that cytokinins are mainly formed in the roots, Cleome spinosa plants were cultured in 0.5, 1.8 and 4 l plastic pots to obtain differently developed root systems. As is shown in Table 8, zeatin stimulated pistil development in flower buds, particularly of plants with reduced root systems.

Table 5: Effect of Ethephon, applied to the leaves at seven-day intervals during 9 weeks, on pistil development. Ten replicates per treatment.

M. Ethephon	0	10^{-5}	3.10^{-5}	LSD (5%)
% female abortion	28.1	23.4	18.9	7.8
inflorescence length (cm)	53	51	53	n.s.[x)]
fruit weight (mg)	137	138	139	n.s.
seed wt./fruit (mg)	38	39	38	n.s.

[x)] n.s. = not significant

Table 6: Effect of Ethephon, applied to de-seeded fruitlets (5 to 11 days after pollination), at seven-day intervals during 6 weeks, on pistil development. Ten replicates per treatment.

M. Ethephon	0	10^{-5}	10^{-4}	10^{-3}	LSD (5%)
% female abortion	29.3	21.5	11.7	11.1	6.6
No. of flowers per plant	193	180	140	111	-
% fruit set	83	84	58	36	-
% flower bud abscission	0	0	6	20	-

Table 7: Effects of kinetin and ABA, applied at seven-day intervals during 9 weeks, on pistil development. Eight replicates per treatment.

Concentration (M)	0	10^{-6}	3.10^{-6}	10^{-5}	3.10^{-5}	10^{-4}	LSD (5%)
kinetin							
to inflorescence	26.4	-	23.7	25.4	26.4	27.8	n.s.[x]
ABA							
to whole plant	22.8	26.1	-	25.5	-	25.4	n.s.
to inflorescence		25.6	-	24.3	-	20.8	n.s.

[x] n.s. = not significant

Table 8: Effect of zeatin (10^{-4}M), applied daily during 5 weeks with a syringe needle at the base of the inflorescence on pistil development.

pot volume	% female abortion		LSD 5%	No. of replicates
	control	zeatin		
0,5 l	56.3	48.5	6.8	24
0,5 l	67.2	52.5	7.1	18
1,8 l	49.5	39.6	5.8	12
4 l	51.0	48.0	n.s.[x]	12

[x] n.s. = not significant

DISCUSSION

The auxin, NAA, and the gibberellins, GA_3 and GA_{4+7}, particularly, increased the abortion of the pistils. Because NAA did not affect fruit development (Tables 1 and 4), its action on pistil development was directly abortive. This conclusion is supported by the observation that local application in deseeded fruitlets had no effect on female abortion, acropetal auxin transport being an unlikely phenomenon after all. However, according to Table 2, NAA possibly affected fruit development in shifting the optimum IAA level to an earlier stage, which might have temporarely changed the competition between fruits and flower buds on the inflorescence. De Jong et al. (1974) showed that high auxin concentrations may, indeed, directly inhibit pistil growth of flower buds grown in vitro. The endogenous auxin concentration may well have been optimum already, female flower parts, particularly, being known to produce auxin in considerable amounts (Hänisch ten Cate et al., 1974).

In spite of the seed-aborting effect of gibberellins, which was shown to reduce pistil abortion (De Jong and Bruinsma, 1974a), gibberellins usually promoted female abortion (Tables 3 and 4). The abortive effect on pistil development was very pronounced in vitro (De Jong and Bruinsma, 1974b). The seed abortion sometimes masked the direct effect of gibberellins on pistil abortion (Table 3), but when seeds were artificially removed the direct adverse effect of gibberellins on pistil development was clearly demonstrated (Fig. 1). Moreover, when applied to deseeded fruitlets, gibberellins failed to affect pistil growth. It was shown earlier, that fruits increase in their gibberellin content only after their effect on female abortion (De Jong and Bruinsma, 1974a). Another feature of the gibberellin treatment was that the weight per seed was not affected, indicating that gibberellins inhibited the initial development of the ovules rather than the further growth of the ovules that escaped the adverse gibberellin effect. This was supported by the fact that a few days after the first gibberellin treatment the fruits already showed elongation, but not yet seed abortion. Thus the abortive effects of gibberellins on pistil and ovule development are very similar.

The ethylene-releasing substance, Ethephon, stimulated pistil growth, without affecting fruit development (Tables 5 and 6), indicating a favourable effect on pistil development. In the experiments in vitro (De Jong and Bruinsma, 1974b), Ethephon only reduced pistil abortion in the presence of gibberellins, which may well be present in the in vivo situation, where gibberellins can be derived from other plant parts.

The results of the present study fit in with data from the literature, mainly derived from plants with unisexual flowers. Weston (1960) with female hop plants, and Champault (1969) with female inflorescences of Mercurialis annua grown in vitro, found auxins to suppress pistil growth. Durand (1967) demonstrated that the beneficial effect of kinetin on pistil development in male flowers of Mercurialis species could be suppressed when kinetin was used together with gibberellin or auxin. Moreover, it is known that zeatin and other cytokinins are potent inducers of pistil development in several plants: Mercurialis (Durand, 1967), Melandrium (Van Nigtevecht, 1969), Luffa acutangula (Bose and Nitsch, 1970), Vitis (Hashizume and Iizuka, 1971). Mohan Ram and Jaiswal (1970) with Cannabis sativa, and Kender and Remaily with Vitis, induced conversion of staminate flowers into pistillate ones with Ethephon. In monoecious plants, such as Cucurbitaceae, gibberellins promote male flowering, whereas auxins and Ethephon induce feminization. The action of auxins is probably largely due to an enhancement of ethylene production (Shannon and De La Guardia, 1969), which has been shown to be an endogenous regulator of sex expression in these plants (Byeers et al., 1972; Rudich et al., 1972a and b). In Cleome, on the contrary, the effect of ethylene on pistil growth is rather indirect, requiring the presence of gibberellins (De Jong and Bruinsma, 1974b).

Although exogenous auxin, like gibberellins, directly reduces pistil development, the main action of endogenous auxin in female abortion seems rather to be an indirect one. The seeds of developing fruits are active producers of IAA, the peak in the IAA level

coinciding with both fruit growth and pistil abortion (De Jong and Bruinsma, 1974a). By increasing the sink activity, IAA enables the fruits to absorb nutrients and cytokinins, the latter being indispensable for pistil development (De Jong et al., 1974). The same authors showed that the occurrence of male flowers cannot be ascribed to lack of nutrients, leaving cytokinin deficiency as the only possibility. Under circumstances unfavourable for pistil development, exogenous zeatin is capable in restoring pistil growth (Table 8). That the female abortion was only partly reduced may rather be ascribed to the high temperature prevailing during experiments and to the presence of gibberellins derived from other plant parts, than to a possibly defective way of application by a needle. Methylene blue, applied in the same way, was found to be evenly distributed over the flower buds. The occurrence of cytokinins in Cleome fruits has not been demonstrated, but fruits are known to absorb cytokinins from the sap stream (Varga and Bruinsma, 1974).

The intermittent formation of perfect and male flowers in zones on the inflorescence of andromonoecious Cleome species, can therefore be ascribed to a lack of cytokinins in flower buds owing to the sink activity of subjacent developing fruits.

ACKNOWLEDGEMENTS

We wish to thank Miss A.C. Burgers, Mrs. J.H. IJff and Mr. A.P. Everaarts for their co-operation. This investigation was supported by the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

REFERENCES

Bose, T.K. and J.P. Nitsch: Chemical alteration of sex-expression in Luffa acutangula. Physiol. Plant. 23, 1206-1211 (1970).

Burrows, W.J. and D.J. Carr: Cytokinins content of pea seeds during their growth and development. Physiol. Plant. 23, 1064-1070 (1970).

Byers, R.E., L.R. Baker, H.M. Sell, R.C. Herner and D.R. Dilley: Ethylene: A natural regulator of sex expression of Cucumis melo L. Proc. Nat. Acad. Sci. 68, 717-720 (1972).

Champault, A.: Masculinisation d'inflorescences femelles de Mercurialis annua L. (2n=16), par culture in vitro, de noeuds isolés en présence d'auxines. C. R. Acad. Sci. Paris 269, 1948-1950 (1968).

Durand, B.: L'Expression du sex chez les mercuriales annuelles. Bull. Soc. Franç. Physiol. Végét. 13, 195-202 (1967).

Hänisch ten Cate, Ch.H., J. Berghoef, A.M.H. van der Hoorn and J. Bruinsma: The abscission of flower bud pedicels in Begonia. IV. Regulation by endogenous hormones. Acta Bot. Neerl. , - (1974).

Hashizume, T. and M. Iizuka: Induction of female organs in male flowers of Vitis species by zeatin and dihydro zeatin. Phytochem. 10, 2653-2655 (1971).

Jong, A.W. de and J. Bruinsma: Pistil development in Cleome flowers. I. Effects of nutrients, development of leaves and fruits on female abortion in Cleome spinosa Jacq. In press (1974a).

Jong, A.W. de and J. Bruinsma: Pistil development in Cleome flowers. III. Effects of hormonal factors on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro. In press (1974b).

Jong, A.W. de, A.L. Smit and J. Bruinsma: Pistil development in Cleome flowers. II. Effects of nutrients on flower buds of Cleome iberidella Welw. ex Oliv. grown in vitro. In press (1974c).

Kender, W.J. and G. Remaily: Regulation of sex expression and seed development in grapes with 2-chloroethylphosphonic acid. HortScience 5, 491-492 (1970).

Knegt, E. and J. Bruinsma: Rapid, sensitive and accurate determination of indolyl-3-acetic acid. Phytochem. 12, 753-756 (1973).

Mohan Ram, H.Y. and V.S. Jaiswal: Induction of female flowers on male plants of Cannabis sativa by Cepha. Experientia 26, 214-216 (1970).

Nigtevecht, G. van: Invloed van een cytokinine op de balanssituatie in de bloemaanleg van Melandrium. Genen en Phaenen 13, 12-14 (1969).

Rudich, J., A.H. Halevy and N. Kedar: Ethylene evolution from cucumber plants as related to sex expression. Plant Physiol. 49, 998-999 (1972a).

- - -: The level of phytohormones in monoecious and gynoecious cucumbers as affected by photoperiod and Ethephon. Plant. Physiol. 50, 585-590 (1972b).

Shannon, S. and M. de la Guardia: Sex expression and the production of ethylene induced by auxin in the cucumber (Cucumis sativus L.). Nature 223, 186 (1969).

Varga, A. and J. Bruinsma: The growth and ripening of tomato fruits at different levels of endogenous cytokinins. J. Hort. Sci. , - (1974).

Weston, E.W.: Changes in sex in the hop caused by plant growth substances. Nature 188, 81-82 (1960).

Ir. A.W. de Jong and Prof.Dr. J. Bruinsma,
Department of Plant Physiology, Agricultural University,
Arboretumlaan 4, Wageningen, The Netherlands.

SAMENVATTING DER ARTIKELEN

I. Effekten van minerale voeding en de invloed van bladeren en vruchten op de vrouwelijke abortie bij Cleome spinosa.

In de inleiding is erop gewezen dat de gehele plant betrokken is bij de vorming van bloemen. Door delen van de plant te verwijderen, kan men een indruk verkrijgen welke invloed dit heeft op de knopontwikkeling. De stamperontwikkeling kan afhangen van voedings- en hormonale faktoren, deze kunnen de stampergroei zowel remmen als bevorderen. Een hoog mineraal voedingsniveau doet het aantal bloemen toenemen en begunstigt de stamperontwikkeling. De aanwezigheid van uitgegroeide bladeren - een bron van fotosynthetische assimilaten - is eveneens gunstig voor de stamperontwikkeling.

De ontwikkeling van jonge vruchten is sterk van invloed op de stampergroei in de hogerliggende bloemknoppen. Deze vruchten accumuleren auxine en gibberellinen. Auxine (IAA) hoopt zich vooral op in de tweede week en gibberellinen nemen eerst tegen het eind van de vruchtrijping geleidelijk toe. Wanneer het niveau van de gibberellinen het hoogst is, is het nadelige effekt van de vruchtgroei op de vrouwelijke abortie reeds voorbij. Stamperabortie kan dus niet worden toegeschreven aan het gibberellinegehalte van de vruchten. De toename van het IAA-gehalte in de zaden der vruchten verloopt parallel aan vruchtgroei en stamperabortie. De stamperabortie kan dus worden toegeschreven aan deze auxineproduktie, hetzij door een direkt effekt van auxine op de stamperontwikkeling, hetzij door een door auxine geïnduceerde concurrentie tussen vruchten en bloemknoppen aan de bloeiwijze om voedings- en hormonale stoffen.

Stamperabortie bij Cleome spinosa kan dan ook voorlopig worden toegeschreven aan de "sink" aktiviteit van zich ontwikkelende vruchten. Deze "sink" aktiviteit ontstaat als gevolg van de auxineproduktie van de zaden.

II. Effekten van voedingsstoffen op de ontwikkeling van *in vitro* gekweekte bloemknoppen van *Cleome iberidella*.

Om na te gaan wat de effekten zijn van voedings- en hormonale stoffen op de bloemvorming, zijn bloemknoppen van *Cleome iberidella* op kunstmatige media gekweekt. In dit hoofdstuk is vooral de invloed van de minerale samenstelling van het medium onderzocht. Zuurgraad, suikergehalte en samenstelling van de stikstof bleken van groot belang te zijn voor de ontwikkeling van de bloemknop. Alleen bij bepaalde nitraatconcentraties stimuleerden lage ammoniumconcentraties de bloemknopontwikkeling. Kroonbladen en stampers reageerden verschillend op een bepaalde voedingsconcentratie. De kroonbladen worden bij afnemende concentraties van organische en anorganische voedingsstoffen reeds geremd, terwijl de stamperontwikkeling nog optimaal blijft. Als dezelfde voedingsafhankelijkheid ook geldt voor *Cleome spinosa* *in vivo* is het onwaarschijnlijk dat de remming van de stamperontwikkeling toegeschreven kan worden aan voedingsgebrek. Gebrek aan voedingsstoffen bij intakte planten zou dan eerder tot kroonbladremming leiden dan tot remming van de stampergroei. De conclusie is dat de ontwikkeling van mannelijke in plaats van tweeslachtige bloemen veroorzaakt wordt door gebrek aan hormonale stoffen als gevolg van de zich ontwikkelende vruchten aan de bloeiwijze.

III. Effekten van regulerende stoffen op de ontwikkeling van *in vitro* gekweekte bloemknoppen van *Cleome iberidella*.

Zonder toediening van regulatoren ontwikkelen zich bij de *in vitro* kweek van bloemknoppen hoofdzakelijk mannelijke bloemen. De cytokininen zeatine en BA zijn de enige groeiregulatoren die de stamperontwikkeling aanzienlijk stimuleren. Deze cytokininen zijn nodig voor de laatste celdelingen in de stamper.

Effekten van andere regulatoren kunnen slechts bestudeerd worden, wanneer cytokininen in het medium aanwezig zijn. NAA stimuleert de stamperontwikkeling bij lagere concentraties en remt deze bij hogere concentraties. Gibberellinen remmen direkt de stampergroei, ditzelfde effekt vertonen zij ook bij de *in vivo* experimenten (IV).

De remming van de stamperontwikkeling door GA_{4+7} wordt door ABA, Ethephon en Chlormequat tegengewerkt. In afwezigheid van gibberellinen remt ABA de stampergroei in sterke, Ethephon in mindere mate, terwijl Chlormequat deze groei nauwelijks beïnvloedt. Dit wijst erop dat ook Chlormequat niet zozeer effekt heeft op de synthese van gibberellinen maar wel op het door gibberellinen geïnduceerde aborterend effekt. Evenmin als ABA en Ethephon, kan Chlormequat de door gibberellinen geïnduceerde groei bij stampers, die ontsnappen aan het aborterend effekt, niet reduceren.

Analyse van de effekten van groeiregulerende faktoren op de knopontwikkeling in vitro leidt tot een hypothese over het mechanisme van de regulering van de geslachtsexpressie bij Cleome-soorten. De ontwikkeling van de stamper vereist specifieke cytokininen voor de laatste celdelingen. Deze celdelingen hebben in de andere bloemdelen reeds plaatsgevonden. Omdat in vivo de cytokininen uit de wortels komen, is het effekt hiervan op de ontwikkeling van de stamper alleen in vitro waar te nemen. Cytokininen, mogelijk bijgestaan door kleine hoeveelheden auxine, zorgen voor de completering van de bloem.

Vrouwelijke abortie, veroorzaakt door zich ontwikkelende vruchten, kan het gevolg zijn van de produktie van gibberelline-achtige hormonen of van een beperking in de aanvoer van voedingsstoffen en cytokininen naar hogerliggende bloemknoppen. De eerste mogelijkheid vervalt omdat het effekt van vruchten op de stamperontwikkeling optimaal is wanneer hun gibberellinegehalte nog zeer laag is (I). Wat de tweede mogelijkheid betreft, is gevonden dat gebrek aan minerale- of organische voedingsstoffen de groei van de stamper minder beïnvloedt dan die van de kroonbladen (II). De laatste mogelijkheid is dat het aborterend effekt van vruchten op de stamperontwikkeling in hogerliggende bloemknoppen aan de bloeiwijze veroorzaakt wordt door concurrentie om cytokininen vanuit de wortels.

IV. Effekten van regulerende stoffen op de vrouwelijke abortie bij Cleome spinosa.

NAA en gibberellinen stimuleren de vrouwelijke abortie. Omdat NAA geen effekt heeft op de vruchtontwikkeling werkt het direkt aborterend. Hierbij komt nog dat toediening in zaadloze vruchten geen effekt heeft op de stampergroei. Door NAA-behandeling verschuift het optimum IAA-niveau naar een vroeger stadium, waardoor de mogelijkheid bestaat dat de concurrentie tussen vruchten en bloemknoppen tijdelijk verandert. Hoge auxineconcentraties kunnen direkt de stampergroei remmen (III).

Hoewel gibberellinen de zaadabortie stimuleren - en hiervan is bekend dat het de stamperabortie reduceert (I) - bevorderen zij de vrouwelijke abortie. Wanneer zaden op kunstmatige wijze uit de vruchten verwijderd worden, is het direkte nadelige effekt van gibberellinen op de stamperontwikkeling veel duidelijker. Toediening van gibberellinen aan zaadloze vruchten heeft geen effekt op de stamperabortie. Dit ondersteunt dus de hypothese, dat de remming van gibberellinen niet via de vruchten verloopt. Het gewicht per zaad wordt niet beïnvloed door gibberellinebehandeling. Dit wijst wel op een nadelig effekt op de zaadknoppen, maar niet op de verdere groei van die zaadknoppen, die ontsnappen aan het aborterend gibberelline-effekt. Er is dus een vergelijkbaar effekt van gibberellinen op de ontwikkeling van stamper en zaadknoppen.

Ethephon heeft een stimulerend effekt op de stampergroei zonder dat het de vruchtgroei beïnvloedt. In de in vitro proeven is alleen een stimulerend effekt van Ethephon waargenomen wanneer tevens gibberellinen in het medium aanwezig waren (III). Een situatie die in vivo ook waarschijnlijk is, en waarbij dan de gibberellinen kunnen worden verkregen van andere plantendelen. Het effekt van ethyleen op de stamperontwikkeling is dus, in tegenstelling tot wat bekend is bij eenhuizige Cucurbitaceae, veeleer indirekt, omdat gibberellinen hierbij nodig zijn.

Alhoewel exogeen auxine, evenals gibberelline, de stampergroei direkt reduceert, schijnt de belangrijkste werking van endogeen

auxine op de vrouwelijke abortie indirekt te zijn, namelijk door vergroting van de "sink" aktiviteit van de vruchten. IAA stelt de vruchten in staat om voedingsstoffen en cytokininen te absorberen. Deze laatsten zijn onmisbaar voor de stamperontwikkeling (II). Exogeen toedienen van zeatine aan planten onder condities die ongunstig zijn voor de stampergroei, geeft een herstel te zien van de stamperontwikkeling.

De conclusie is dat het verschijnsel, dat afwisselend tweeslachtige en mannelijke bloemen aan de bloeiwijze van Cleome worden gevormd, kan worden toegeschreven aan het tekort aan cytokininen in de bloemknoppen boven de zich tegelijkertijd ontwikkelende vruchten.

SLOTWOORD

Als afsluiting van dit proefschrift wil ik graag allen bedanken die mij hun medewerking hebben gegeven.

Dit geldt in de eerste plaats mijn promotor, Prof.Dr. J. Bruinsma, die met zijn voortdurende belangstelling en waardevolle suggesties een grote stimulans voor mij was. Verder vermeld ik graag de wetenschappelijke medewerkers van de afdelingen Plantenfysiologie en Plantkunde. Zonder hen had het mij heel wat meer tijd en moeite gekost om dit proefschrift af te ronden.

Mej. A.C. Burgers en Mevr. J.H. Yff-Wolters hebben mij steeds ijverig en opgewekt ter zijde gestaan. Ook de samenwerking met het zevental studenten dat gedurende kortere of langere tijd aan mijn onderzoek deelnam, heeft veel voldoening gegeven.

Veel waardering heb ik voor de grote zorg waarmee de heren J. Verburg en G. van Geerenstein het vele plantmateriaal opkweekten. De heer R. van der Laan wist steeds een oplossing te vinden voor de technische problemen. De heren W. Scheepmaker en T. Zaal hebben het teken- en fotowerk voor hun rekening genomen. Mevr. J.W. Tak-Vis verzorgde het vele typewerk.

Zeer erkentelijk ben ik de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek die mij in staat heeft gesteld dit onderzoek te doen.

CURRICULUM VITAE

Na zijn studie in de plantenveredeling aan de Landbouwhogeschool te Wageningen in de jaren 1962-1970 werd de auteur, als onderzoekmedewerker door de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek gedetacheerd op de Afdeling Plantenfysiologie van de Landbouwhogeschool.